

No market for US education

Not since 1957 (Sputnik year) has there been such a general agreement that high-school education in the United States is in a mess. Sooner rather than later, the administration will have to intervene.

THE Reagan Administration is showing every sign of having bitten off more than it is willing to chew by its decision in 1981 to establish a national commission to review education standards in high schools. The commission's final report was expected to confirm that all was not well but not to go so far as to proclaim the nation "at risk" and to advocate a crusade of expensive reforms such as raising teachers' pay and lengthening the school day. Now that it has, with two quite separate reports from the Twentieth Century Fund and the Education Commission of the States bearing equally bad tidings, the question is whether public opinion has received enough of a jolt to compel the administration to accept the responsibilities the reformers want to give it.

So far, President Reagan appears unwilling to abandon his belief that the best thing the federal government can do for education is to have nothing to do with it. The President maintains that education is a state, local and ultimately family affair with which big government ought not to meddle. His administration remains technically committed to abolition of the Department of Education, although congressional hostility and bureaucratic footdragging make that a distant, even improbable, prospect. The only other major features of the administration's educational policy are its desire to return prayer (outlawed by the Supreme Court) to the public (free) schools and to introduce a system of tax credits for parents who send their children to fee-paying schools.

This flimsy agenda is unlikely to survive the tempest of public alarm generated by the latest trio of reports. All contend that the deterioration of educational standards has gone beyond the point at which the states and districts can be expected to retrieve the situation without a strong dose of national leadership. The national commission puts responsibility for protecting the national interest in education and implementing the necessary reforms squarely on the shoulders of the federal government. The Twentieth Century Fund says that only a "major federal initiative" could galvanize reform on the scale that is needed.

Local control

All this must, however, be taken with a pinch of salt. The most important and inescapable fact about American schooling is that it is highly decentralized, with control of the curriculum vested in some 16,000 school districts and control of the purse-strings in the hands of localities and states, with the federal government coming a poor third. None of this is likely to change, so intervention by the federal government has to consist of delicate touches on the rudder, not clumsy attempts to change the overall direction. With this said, what form should the delicate touches take? Here, the national commission report and its companions give too little guidance.

There has not been much doubt, in recent years, about the reason for the decline in educational standards. There are three interconnected causes. The first is a change in what might be called the "culture" of schooling, a phenomenon reflected in the reports by complaints about the failure of teachers to demand high standards from their students or to set enough homework. The second is a dilution of the academic content of the curriculum and a tendency to permit too much time to be squandered on popular elective subjects (auto repairs, driving lessons, sport) and not enough on mathematics, language and science. The third, and

perhaps the most important, is the deterioration of the teaching force. Reform, it follows, must consist of harder work, a more rigorous focus on basic academic subjects and better teachers. The difficult question is not what should be done but who is capable of doing it.

It is not within the gift of governments to make students work harder or to change the culture of the schools; not, that is to say, in plural and dynamic societies such as the United States. Children permitted to watch hours of television every night will not attend to their homework unless grave penalties are attached to a failure to do so. Grave penalties will not be condoned by parents accustomed to treating their children as diminutive equals free to make their own choices and set their own priorities. But the culture of the school is susceptible to subtle manipulation by changes in the curriculum. Here, it seems, changes are already in progress. Alarmed by the amount of remedial work their freshmen and women appear to need, universities throughout the United States have in the last two or three years begun to raise their entrance standards, and the new requirements have begun to percolate through the high schools. Students knocking at the gates of decent universities will not in future be allowed in unless they have demonstrated basic competence in mathematics, English, a foreign language and, increasingly, some understanding of computers.

Motivation

Changes in the atmosphere of schools and the desire of children to learn take place gradually and are generally beyond the control of the federal government. What the federal government can and should do is make sure that there are good enough teachers in the schools to exploit these changes in mood and motive. The quality of teachers at present is dismal and shows signs of getting worse. New teachers are being drawn from the bottom quarter of high school graduates. Too few are capable of passing the ludicrously simple teacher competency test and only a trickle master the more difficult subjects such as mathematics and science.

The essential reasons for the flight from teaching are low pay and the low prestige that goes with it. After a dozen years in the classroom, the average teacher will earn \$17,000, not much more than the starting salary of a city policeman. Pushing up the overall pay of the teaching profession to a competitive level at which, say, \$40,000 or more was available for good and experienced teachers, would be beyond the means of most states and school districts. There is, however, another way. In recent months a number of states have realized that if the teaching profession were pyramidically structured, so that only those who really proved their worth in the classroom were allowed to rise in pay and status, it would be possible to create an elite group of "master teachers" earning competitive salaries. Such a development would make teaching a genuinely attractive option for bright young students confident of their ability to rise through the ranks.

The obstacle is the teachers' unions. An ambitious plan to pump more money into the schools in Tennessee in return for the creation of a "master teacher" career track was scotched two months ago by opposition from the National Education Association. The American Federation of Teachers, similarly, opposes the notion of merit pay on the unpersuasive grounds that such measures divide the profession and create an atmosphere of com-



petition inimical to good teaching. The opposition of the unions ought to be circumvented, and it is only the federal government which can do so. The most effective of all the measures advocated by the three recent reports is a suggestion by the Twentieth Century Fund that the federal government itself create a master teacher fund to encourage the unions to relax their opposition.

The evidence of crisis within the schools has become too clear for the administration to persevere in its belief that the problems can be left for others to solve. What is important is that federal intervention should not be dissipated on too many initiatives, as in the past. A measure which draws a good number of talented young teachers into the schools is long overdue. □

Students on the streets

The French Government should have seen the past weeks' trouble coming.

TEAR gas, barricades and batons are the fashions in Paris this spring, just as they were in 1968. And, now as then, the universities are the focus of all the trouble. For while the universities may be over-politicized, inward-looking and conservative, politicians and the newspapers take them very seriously. Where else but in France would a parliament devote a whole week to a bill reforming higher education, as the French National Assembly will do next week? But 1983 is the inverse of 1968. The trouble then precipitated debate and led to reform, but the 1983 riots are directed against a proposed reform and may even stifle debate. One member of the council of the University of Paris (VII) was right to say last week that the voices shouting in the streets these past few weeks are biased, either left or right. People supporting one aspect of the bill are marching side by side with those who are against it. The student leadership is divided at least three ways. The riots are in every sense a mess and are as much political as academic.

Amid all the clamour, proper debate of the bill's provisions is being neglected. The best hope now is that next week's meeting of the Assembly will rise above the squabbling, for the bill's objectives deserve a careful, even a sympathetic, consideration. Thus the bill would integrate into the French system of higher education the élite *grandes écoles* which Faure left alone in 1968. For individual universities, the bill reaffirms the three principles of 1968 of autonomy, multidisciplinary and democracy and the Mitterrand Government shares with others elsewhere the ambition to gear the universities (and their research) into the economy. The bill also aims to make universities more responsive to their students (including the 40 per cent who now "drop out") and their ultimate employers and to loosen the "complex and rigid" structures of higher education in France. The triple premise of the bill is that 1968 was a good thing, that many of the Faure reforms were never properly carried through or have been forgotten and that in 1983 a prime objective must be to make the intellectual power of the universities work for the nation. All this is laudable, even stimulating, but what does it mean?

The French Government's notion of university autonomy, for example, is not that understood elsewhere. The meagre autonomy granted to French university presidents in 1968 has since been whittled away until it has dwindled almost to nothing. The ministry, now as before 1968, controls the details of university budgets and — advised by a national commission — appoints their staffs. Elephantine committees within the universities hinder management. One objective of the bill is a loosening that may, in France, feel like autonomy but will still look like central control in, say, the United States. Budgets will be provided by multi-annual "contracts" with the ministry, allowing greater local freedom. Staff selection will be essentially local but appointments must be approved by a central committee. And large internal committees will remain but be made more responsive (it is claimed). Moreover, university degrees will continue to be granted by the nation, not by the university, so that the nature of individual courses, at every level, will be determined nationally, by interministerial commission.

Oddly enough, the protests of the past few weeks have not been about autonomy: what little the bill grants will be gratefully received. The protests concern things like a perceived threat to a national institution, the *baccalauréat*, and the possibility of examinations to progress from the first to the second stage of studies (*premier cycle* to *deuxième*). Here the bill makes a political error in being simultaneously explicit and vague. Take *le bac*, for example, the high school qualification that has been the entry ticket to the universities. Article 12 of the bill opens another route to the *premier cycle* (or first stage) of the universities: it will be enough to "have obtained an equivalent qualification" or a dispensation on the basis of experience "judged satisfactory". The government has many times said that it wishes to open the universities to wider social classes (only around one in eight French university students are children of workers for example), so this clause is feared as an abandonment of the sacred institution of the *bac*, an opening of the flood gates. However, many universities already admit non-*bac* entrants, through special courses. The bill merely rationalizes a fact.

The same political error of being both explicit and vague has confused the issue of the proposed examinations to enter the *deuxième cycle*. The bill says (Article 13) that for "certain studies" entry to the *deuxième cycle* might be by success at an examination or an assessment of past work. But there is already *de facto* and severe selection at the second stage, where the universities weed out the many first stage hangers-on. The bill errs again only in saying so in black and white and then being doubly vague — "certain studies . . . might" be by examination. The intention seems to be that these uncertainties should be cleared up by decrees not yet promulgated, but in the meantime there is fertile soil for doubt. A more rational criticism would be to point out that Article 13 suggests that the numbers allowed to enter a *deuxième cycle* will take account of likely future employment in the professions covered by the degree. Whose crystal ball will the ministry be using to determine the figure? Such planning has been tried before, and usually proved impossible.

Manpower planning of a kind has also provoked the troubles which, in the past several weeks, have brought medical students onto the streets and which have driven hospital doctors to strike. In a quite separate piece of legislation enacted last December, the Mitterrand Government has tried to grasp an obvious nettle — the overproduction of physicians by the teaching hospitals — without seeming to care that the career expectations of would-be physicians were being altered drastically. Traditionally, however, hospital interns have put up with miserable stipends in the belief that personal prosperity would follow when they had done their time. Similarly, medical students embarked on traditional courses have been offended by the proposal that the transition from one cycle to another should be determined by performance in tougher mid-term examinations. Next week, it should be clear just what the government intends by its promise two weeks ago that these measures would not apply to those already embarked on studies.

Next week it should also become clear how the French Government plans to fill the obvious gaps in its programme for the reform of the university system as a whole. For most academics, the grand phrases of the bill to be debated next week will be less important than the fine print of the decrees not yet published. The obvious danger is that the Mitterrand Government's grand recipe for reform will be undermined by what will come to seem to the government to be its own responsibility for making sure that its dreams come true. For the mechanisms of central control will survive whatever changes are agreed in the Assembly, as will the temptation of the government to interfere with what the universities are about whenever some public ill can be laid at the door of higher education. The argument about the status of the *bac* in the past few weeks has obscured the whole question of what the university as such is for — higher education primarily, a contribution to the national economy on that account and an institution that can contribute to industrial innovation by the intelligent use of resources in research and on vocational and continuing education. The French Government risks putting its priorities in the wrong order. □

US defence policy

Anti-satellite weapon still under fire

Washington

THE Reagan Administration is coming under mounting congressional pressure to postpone tests planned for this summer on the United States' first air-launched anti-satellite weapon. Four resolutions have been submitted in the House of Representatives and the Senate calling for the United States and the Soviet Union to resume talks on banning all weapons from space. Their sponsors claim that deployment of the new weapon, a miniature homing vehicle launched from under the wing of an F-15 aircraft, would make verification of such a treaty virtually impossible.

Talks on the banning of anti-satellite weapons were broken off by the Carter Administration in 1979 after the Soviet intervention in Afghanistan. In 1981, the Soviet Union submitted to the United Nations a draft treaty banning the stationing of weapons in space, but the United States has not yet decided whether to resume negotiations. The administration's interim policy is to support the principle of banning space weapons while drawing attention to the fact that the Soviet Union is believed to possess the world's only operational anti-satellite system.

Department of Defense sources claim that the Soviet system, based on launching an explosive satellite interceptor from the ground, has been tested on 20 occasions. The department believes the Soviet Union might be capable of placing a laser weapon in space within a few years. The Soviet Union's apparent advantage in anti-satellite weaponry is cited by the administration as the principal justification for its development of the air-launched system.

At a press conference last week, however, Representative Joseph Moakley (Democrat, Massachusetts) said that deployment of the US weapon would leapfrog Soviet technology and represent an "irreversible step" towards a space arms race. Moakley, who has the support of some 120 congressmen for a resolution banning space weapons, said the small size of the American weapon and its ability to be launched without warning from an ordinary fighter aircraft would pose a verification "nightmare". Once it was operational, he said, the Soviet Union was unlikely to enter any kind of agreement banning space weapons.

Two live firings of the new weapon are planned this summer and the President's 1984 budget request for the programme includes \$19 million for procurement — the first time procurement funds have been requested for the anti-satellite programme. Current estimates are that the system could be operational in 1987.

Representative Moakley's resolution is receiving powerful backing from many of the US scientists who played a big part in the development of ballistic missiles. Dr Richard Garwin, a long-serving Defense Department consultant who helped design multiple independent re-entry vehicles for

missiles (MIRVs), said that the deployment of anti-satellite weapons could lead to a pre-emptive war in space during times of peace.

Earlier this year, Dr Garwin and Professor Carl Sagan organized a petition calling on all "spacefaring" nations to outlaw weapons from space. Signatories included two former presidential science advisers and a large number of former Pentagon officials. The scientists received a reply last month from Yuri Andropov reaffirming the Soviet desire to resume talks on a space ban treaty.

Peter David

Unclassified secrets

Stanford protests at data freeze

Washington

THE Department of Energy (DoE) is proposing to clamp down on unclassified information relating to nuclear weapons, a move that universities say would have a chilling effect on fundamental research supported by DoE.

The proposal — which stems from a 1982 law allowing the department to "protect" information that could help terrorists to make nuclear weapons, steal nuclear material or sabotage nuclear facilities — would establish a new category of "unclassified controlled nuclear information" that could be released only to government officials or government contractors having a "need to know". Under the rule, government contractors — presumably including universities — would have to establish procedures that would "at a minimum" treat such information with the level of secrecy "customarily accorded proprietary business information".

The rule describes in general terms the sorts of data that are to be considered "controlled". Information on the design and manufacture of nuclear weapons is the most important; the rule specifically notes that this is information that was at one time classified but was "subsequently declassified at a time when . . . [terrorism] was a relatively infrequent occurrence and when nuclear proliferation was not a serious threat". Information concerning naval nuclear propulsion, security around nuclear defence facilities, and nuclear material production and processing would also be covered, if its release could be "inimical" to the "common defense and security" of the country.

But the proposal is vague when it comes to how someone in possession of such unclassified material is to know that it is considered "controlled". This problem in particular prompted Stanford University's dean for graduate studies and research, Gerald Lieberman, to lodge a strong protest in a letter to DoE. He argues that the rule has an "unlimited potential to chill research, teaching, and the general interchange of information . . . It seems to apply to any information, no matter when pro-

duced or by whom, so long as it was 'ever developed by or for' the United States Government". Lieberman wrote that Stanford would be unable and unwilling to search all of its documents for material that might fit the rule's definition.

He also asserted that the new rule could end up preventing Stanford and other institutions that forbid classified research on campus from taking on any DoE contracts. Stanford's policy is not to accept any research from sponsors who place restrictions on publication. "The DoE rule provides no assurance that results will be distributable", he wrote; a researcher may not even be able to know until he has done the research that its results would be considered "controlled".

Lieberman added in an interview: "In a way, we argue — please classify it, so we'll know where we stand. We're troubled by being in this never-never land of not knowing."

Trisha Chico of DoE's Office of Defense Programs said the agency has no intention of requiring a "wholesale review of documents" already published and on library shelves. Only documents reviewed by a designated DoE official and found to meet the rule's criteria would have to be secured and stamped with a warning.

But she did suggest that the intention of the rule was that if a DoE contractor develops new information in the course of a DoE project, or receives a request for information from a non-authorized person, and that information is a "likely candidate" for being controlled, the contractor would be expected to send it to DoE for review before releasing it. She added that the rule was unlikely to have much effect on an institution such as Stanford, since places that are not defence-related "are not going to have much of that information".

Several institutions that could be affected by the rule learned of it only in the past two weeks, and DoE has agreed to extend the comment period for 30 days, until 3 June. The original proposal appeared in the *Federal Register* on 1 April (p. 13988).

Stephen Budiansky

Foot and mouth disease

Australian lab a white elephant?

Canberra

THE future of a A\$150 million laboratory for the study of foot and mouth disease virus has been thrown into confusion. Although the facility is now virtually complete, the federal government is considering a report by the Australian Science and Technology Council (ASTEC), tabled in the House of Representatives on 5 May, recommending that live foot and mouth disease virus should not be imported into Australia for a period of five years. ASTEC reports directly to the Prime Minister, and the recommendation is part of its inquiry into the Australian National Animal Health Laboratory (ANAH), a high-security microbiological facility scheduled for completion later this year, as part of CSIRO's Division of Animal Health.

Importation of live virus for any purpose has long been a vexing issue. Australia, because of its geographical isolation, is free from many animal diseases prevalent elsewhere. The fact that it has remained so, despite the advent of air travel, is testimony to the effectiveness of strict quarantine regulations. By far the most serious threat is the foot and mouth virus because a single outbreak would mean the immediate closure of US and Japanese markets, with trade resuming only 6–12 months after its eradication was proved, with a loss of some A\$2,000–3,000 million.

In order to be able to cope with an outbreak of an exotic disease such as foot and mouth, ANAH was approved in 1974 by the Parliamentary Standing Committee of Public Works, after the need for such a facility had been urged by the Australian Agricultural Council. Because of the reluctance of the government of the day to commit itself to the large investment required, construction was postponed until 1978. In the meanwhile, capital costs had escalated from A\$67 million to the current estimate of A\$150 million, with an operating budget of A\$7 million per year.

While there is little doubt that live virus would be needed after an outbreak, and in any case, would be available from the field, controversy surrounds the question of whether it will be needed beforehand. CSIRO maintains that live virus is necessary for (1) training of personnel to recognize the clinical symptoms of the disease in all its manifestations, (2) vaccine production to combat its spread and (3) positive controls in diagnostic tests to guard against a false negative.

However, a recent report by the Australian Academy of Science systematically demolishes these arguments — personnel can be trained overseas; slaughter and not vaccination ahead of the moving front of infection will be the easiest and quickest line of defence against a primary outbreak; using inactivated antigens and

developing new diagnostic methods can unequivocally establish the causative agent. In any case, action must be initiated from clinical symptoms without waiting for a laboratory diagnosis.

The argument against the importation of live virus is the danger of its escape, although the security of ANAH is not in question. According to the ASTEC report, "notwithstanding the excellent level of containment, the importation of an exotic

pathogen represents an additional risk over and above that posed by its accidental or deliberate introduction by other means". Nevertheless, the ASTEC report does not unequivocally rule out the need to work with live virus. It recommends the setting up of a small research group in an overseas laboratory with access to live virus, presumably at Pirbright in the United Kingdom, the world reference laboratory for foot and mouth disease virus.

Far from defusing the issue, the ASTEC report focuses attention on the justification for a laboratory primarily designed for research into foot and mouth virus.

Vimala Sarma

In vitro fertilization

BMA reports on ethics

THE British Medical Association (BMA) has published interim ethical guidelines for the medical profession on human *in vitro* fertilization and embryo replacement. The guidelines approve the therapeutic use of the new techniques, including "observations" on fertilized ova in excess of those needed for replacement or transfer.

The report, produced by a working group under Professor Peter Quilliam, was approved by BMA's council on 4 May. Although not formally binding on members of the profession, it will be the most influential of the recent spate of ethical reports on *in vitro* fertilization offered to the government's Warnock Committee.

The report says that *in vitro* fertilization should be carried out only at "special centres" where appropriate facilities and expertise are available. It is therefore unlikely that *in vitro* fertilization will be carried out in the majority of hospitals in the near future. BMA does not, however, follow the Royal College of Obstetricians and Gynaecologists (RCOG) in calling for legislation to license premises where the technique is used (see *Nature* 28 April, p.739), although it demands a system for recording all attempts to secure pregnancy.

The treatment of infertility by *in vitro* fertilization should be preceded by an assessment of the "stability and sincerity" of the couple concerned, the report says. It approves the use of ova or sperm donated by a third party when one of the partners is unable to produce viable gametes, but is less sure about the (rare) case where neither partner is able to produce viable gametes. BMA is unwilling to sanction "surrogate motherhood" — the transferring of an embryo to the uterus of a woman who might bear a child on behalf of an infertile couple.

The guidelines say it is "proper and important" that steps be taken to ensure the effectiveness of *in vitro* fertilization and embryo replacement. To this end, observation of embryos in excess of those needed for replacement will add to medical knowledge. However, such observations should normally be completed within 5 to 10 days of fertilization and always within

14 days. This condition is more restrictive than that urged by RCOG (which recommended 17 days as the end point for research), and is at variance with the recommendations of the Royal Society, which advocated "flexibility".

BMA's recommendations, which scrupulously avoid using the word "experiment", follow those of other groups in insisting that embryos which have been subject to manipulations should not be replaced in a uterus. BMA endorses provisional guidelines published last year by the Medical Research Council (MRC), which say that research on embryos is acceptable if "clearly defined and directly relevant to clinical problems". Dr Anne McLaren, of University College London, who was a member of both the MRC and the BMA working groups, says that the relevance of research proposals would be assessed by local ethical committees of university teaching hospitals (where it is expected that the research would be carried out). A spokeswoman for BMA offered the view that observations aimed at quality control of embryos were probably acceptable, whereas experiments which involved testing hypotheses were less likely to be approved. BMA's guidelines allow the storage of embryos by freezing, if observations show this to be safe; storage should not, however, exceed 12 months.

Two proposed amendments were defeated at BMA's council meeting: one would have prohibited any form of experimentation on fertilized ova, and the other would have made it unethical for a BMA member to cooperate on embryo research with any person not covered by the guidelines.

Taken together, BMA's guidelines and those of MRC will effectively determine what research is done on human embryos (although both are provisional), at least until the Warnock Committee reports next year. The surgery to obtain human ova can only be carried out by a registered medical practitioner, while most non-medical scientists working in the area are supported by the Medical Research Council.

Tim Beardsley

Laboratory animals

Replacement for 1876 act in sight

JUST one day before Parliament was dissolved last week to clear the way for the general election, the British Government published its long-awaited proposals for new legislation on animal experimentation. Opponents of animal experimentation will not be placated — nothing short of a ban on the use of animals, at least in cosmetics testing, would satisfy the antivivisectionists. But the White Paper does take account of the public concern for animal welfare and the practical needs of researchers by stressing a preference for "non-sentient" alternatives wherever possible, and by being specially stringent when licensing cosmetics testing.

The new proposals differ from the current Cruelty to Animals Act of 1876 in several important ways:

- The present law covers only "experiments", but the new law would include procedures such as production of antisera, passaging of tumours and interference with the embryo or fetus.
- A new Animal Procedures Committee would be established by statute to replace the existing non-statutory body. The committee would advise the Home Secretary on matters of special concern and would advise on the development of alternatives to animals.
- All animals used in laboratories would have to be obtained from registered establishments. The use of stray cats and dogs would not be allowed.
- The conditions in which animals are kept when not being used in experiments would be regulated.
- Home Office inspectors would have to be satisfied that a procedure is justifiable, that no satisfactory alternative to the use of animals can be found, that the minimum possible number of animals is used and that the least possible suffering is caused.
- The 1876 act requires all animals used in experiments in which the animal recovers from anaesthesia to be killed. Recognizing that many surgical procedures are now relatively mild and that anaesthesia may also be used for immobilization rather than to control pain, this requirement would be dropped.
- Experiments on anaesthetized animals to train surgeons in microsurgery procedures would be permitted.

One result of the new legislation would be to allow the government to ratify the Council of Europe's "Convention for the protection of animals used for scientific purposes", details of which were settled

THE White Paper Scientific Procedures on Living Animals is published as Command 8883 by HMSO at a price of £3.60. In it, readers are invited to send written comments on the proposals to the Home Office, E4 Division, Queen Anne's Gate, London SW1H 9AT by 12 August 1983. □

two weeks ago. In certain areas, the Home Office claims, the White Paper would go much further than the minimum requirements of the convention. For instance, no exceptions to the "pain condition" would be permitted in the United Kingdom. This means a continuation of the present rule that "if an animal is found to be suffering severe pain which is likely to endure, it shall at once be painlessly killed".

To implement the new legislation, the Home Office would almost certainly have to increase the number of inspectors from

the present level of 15. The Home Office intends to make as many as four inspections each year for every licence holder, in contrast with one visit every four years which is normal in some other European countries.

Publication of the new proposals is a step towards keeping one of the promises made in the last Conservative manifesto to "update the legislation on experiments on living animals". If a Conservative government is returned on 9 June, it is committed to introducing the new bill into Parliament "as soon as parliamentary time permits". An administration of a different political hue, however, might wish to start with a clean sheet rather than modify the White Paper, and would have many other calls on its parliamentary time. **Charles Wenz**

US advisory committees

Same jobs, but less pay

Washington

FEDERAL agencies may have to stop paying consulting fees to scientists who serve on peer review panels under a new rule issued late last month by the General Services Administration. The rule, which affects advisory committees throughout the government — including some 200 committees that review grant proposals for the National Institutes of Health (NIH) and the National Science Foundation (NSF) — was issued as part of the Reagan Administration's effort to foster "volunteerism" as a substitute for government spending.

The administration "believes that a sufficient number of citizens of all backgrounds and qualifications can be found to provide advice . . . through voluntary service", the rule states (*Federal Register* 28 April, p.19324).

Agencies would still be permitted to pay transportation expenses for committee members and a *per diem*. But, according to NIH and NSF officials, the *per diem* — \$75 per day for Washington — is not enough to cover hotel and meal expenses. Both agencies have been paying their advisers consulting fees of \$100 per day for the time they spend at committee meetings.

The agency officials noted that this is far below consulting fees paid by industry, and in fact is also far below the \$245 per day that they could have paid under the previous rules. Vida Beaven, special assistant to the NIH deputy director, added that their advisers put in "as much as two to three weeks of homework time" before the thrice annual meetings for which they receive no pay.

NIH and NSF are exploring the possibility of obtaining an exemption to the rule, and for the present are continuing their past policy. The new rule does allow compensation for advisers if the law authorizing the committee specifically calls for it; it also allows compensation in the "exceptional case" where the agency head is unable to obtain the required expertise or balanced representation on the commit-

tees. But this latter exemption can only be invoked on a case-by-case basis, and apparently would not allow the NIH or NSF director to declare a blanket exemption for the entire agency. And although counsels for the agencies are examining the authorizing legislation for their committees, it seems doubtful at first glance that they will be able to claim specific legislative authority for paying advisers.

Nobody seems to have any firm data on just what effect the rule will have. "We're not saying that NSF will go under and everybody will resign", conceded Jeff Fenstermacher, director of NSF's division of personnel and management. "But we think [\$100 per day] is little enough to pay for all the work they do for us. You'd be asking them to pay money out of their own pocket to do work for the government."

Fenstermacher said that one worry was that the rule could foster the "old boy" network by encouraging relatively established and well-to-do scientists to serve on the peer review panels while discouraging younger researchers. The peer review system is often criticized already on this score. Fenstermacher added that the total cost of consulting fees to NSF last year was \$270,000 — about 1/200th of one per cent of the NSF budget.

The agencies had expected the rule to be issued in the form of a proposal, with a 90 day period for comments. Instead the Office of Management and Budget waived the comment period requirement and allowed the rule to be issued as an "interim" regulation effective immediately. Comments will still be accepted, and may have an effect on the final rule, but the action "gives us some indication of how serious they are", Fenstermacher said.

The rule allows advisers appointed before 15 May to continue to be paid until 30 September. NIH at least will not have to take any immediate steps until 1 July, when nearly a quarter of its 2,400 advisers' terms expire. **Stephen Budiansky**

Academic publishing

New brooms galore

AFTER a turbulent year, Academic Press London (APL), a leading publisher of scientific books and journals, seems temporarily at least to be in calmer waters. This year has seen the "moving" of one managing director, the abrupt dismissal of another and the resignation of several members of the board, but staff concern was reduced last month with the appointment of Peter Jovanovich as managing director.

APL is a wholly owned subsidiary of Academic Press New York, producing about a third of the joint annual output of 600–700 books and 170 journals. The two companies are in turn owned by Harcourt Brace Jovanovich (HBJ), a Fortune 500 company with diverse interests in book and magazine publishing, and marine parks. President and chief executive of HBJ is William Jovanovich, father of Peter Jovanovich. So the new managing director of APL is likely to have the ear of the parent company's board.

The internal problems for APL began in April 1982 with the "removal" of Roger Farrand as managing director (he remains with the company as a consultant) and appointment by HBJ of George Fleming, previously head of HBJ's international division. According to several sources, the reason behind Fleming's appointment was not concern over APL's profitability but imposition on the London office of the systems of management, accounting and distribution used in the United States. "They just didn't like Roger's style" said

one former employee.

Academic Press in New York is itself undergoing a period of change, journals' production having already moved from the east coast to San Diego. Decentralization will be taken further when the books' arm of the company is relocated in Orlando, Florida, next year.

Fleming's methods of management — employees speak of "vindictiveness", "back-biting" and "lack of loyalty" — and reported obsession with redecoration of APL's offices resulted in the steady departure of staff. Most notable among those to leave were Anthony Watkinson, editorial director, and Barbara Wheeler, the personnel director. All departments suffered losses of staff at an unusually high rate, the department responsible for promotion being most severely affected.

Matters came to head last month with further resignations and the publication of an attack on Fleming's management and lavish personal life-style in *Private Eye*, the London-based satirical magazine. Fleming was subsequently fired.

Peter Jovanovich's brief is to concentrate on editorial and production — "the heart and lungs of publishing" — and to restore morale at the company. "I'm not a systems' man" he says. While Jovanovich's honeymoon period is not yet over, a former senior employee was cautiously optimistic about his prospects of success. "He's a nice chap. APL has had an unhappy time, but I think it's now on the mend."

Tim Lincoln

US archaeology grants

An historic victory

Washington

FACED with a storm of protest from archaeologists, the National Science Foundation (NSF) has backed down on a proposal that would have required review of archaeology grant proposals by state government officials.

At a meeting in Pittsburgh with the executive board of the Society for American Archeology (SAA), NSF officials agreed to a watered-down rule that will require archaeologists submitting grant proposals merely to show that they have made contact with the state historic preservation officer for the state in which the research is to be done.

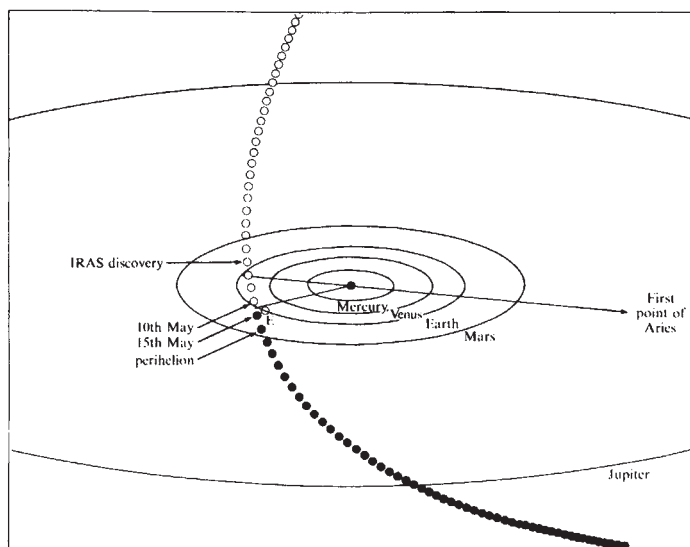
"The first draft was an absolute disaster from the point of view of research", said John Speth, an archaeologist at the University of Michigan. Speth, whose views are typical of the outrage created by the NSF proposal, was particularly critical of allowing the state officials — political appointees, many of whom have no training at all in archaeology — to pass on decisions that should be made solely on scientific grounds. The original proposal would have required the state officials to comment specifically on the cost-effectiveness of the research, the appropriateness of the methods and techniques to be used and the importance of the research to the state's archaeology plan, and would have added another layer of bureaucracy — and more delays — to the grant approval process.

According to government officials, however, the proposal was merely an attempt to comply with the law. Under the National Historic Preservation Act of 1966, any federal action that affects historic sites must be submitted for review to a federal Advisory Council on Historic Preservation. According to Thomas King of the council, NSF has brought only occasional cases to the council's attention.

The archaeologists' outrage may have been fuelled by a recent fight with the council over its proposed rule establishing procedures to survey and excavate archaeological sites on lands for which the Department of the Interior issues strip-mining permits. On this issue, the archaeologists were on the other side of the fence, pushing for tougher oversight by the council, and there is considerable suspicion that the NSF rule was intended to take some heat off the strip-mining fights.

Both issues have now apparently been resolved to the archaeologists' satisfaction — although King is not so sure: "I doubt if they'll really calm down, because fundamentally they don't feel that they should be covered by the law. Well, tell it to Congress."

Stephen Budiansky



THE orbit of Comet IRAS-Araki-Alcock (1983d), computed from observations since its initial discovery on 26 April. The closest approach of the comet to the Earth (about 5 million kilometres) was at midday on 11 May. E marks the position of the Earth at that point. The individual circles mark the comet's position at 5-day inter-

vals, the open circles marking the portion of the orbit above the ecliptic plane of the Solar System. The system is portrayed as seen by a viewer at ecliptic longitude 285°, latitude +20°. The comet's closest approach to the Sun occurs on 25 May. (Picture supplied by N. Eaton, University of Leicester.) Philip Campbell

French research budgets

Let the good times stop

AFTER months of hanging on a thread, French scientists have at last heard how much money they are really getting in 1983. Or at least the research councils have. The Centre National de la Recherche Scientifique (CNRS), the principal basic research council, loses 12.5 per cent of the cash it was granted in 1983 for laboratory expenses — materials, apparatus, special projects and so on. The medical research council (INSERM) loses 10 per cent, and the agricultural research council a little more. One surprising cut: the biotechnology programme of the ministry of industry and research, one of ex-minister Chevènement's incentive "*programmes mobilisateurs*", is slashed by a quarter.

Now it is up to the scientists in each institution to work out how to share out the shrunken cake. At CNRS, for example, the Paris-based scientific directors who manage the different sectors of CNRS research must decide whether to cut general laboratory budgets, or the special programmes (the *actions thématiques programmées*, or ATP), and in what proportion.

But how severe a blow are these cuts, after two years of large increases? Professor Pierre Chambon, director of a prominent laboratory of molecular biology in Strasbourg, calculates roughly that his budget will be 15 per cent down on what he

was promised in January. "And this was the first year in which we really got what we needed", he said on Monday. "We will just have to stop work in November." His losses arise this way: of expected running expenses this year of FF 8.4 million (£720,000), FF 6.5 million comes from basic support by CNRS and INSERM. The rest comes from 2–3 year contracts (such as the ATP and the ministry's biotechnology programme). If the cuts are applied to him in full, Chambon will lose some FF 700,000 on the basic CNRS and INSERM support, and 10 per cent of the remainder as there are likely to be fewer contracts around this year than he expected. That is a loss of around 10 per cent on the total. Then the dollar and other foreign currencies have strengthened against the franc over the past year, and around half of Chambon's budget buys material and equipment from abroad.

However, the loss was on a good budget, so Chambon believes his laboratory is still 5–8 per cent up in real terms on its position in 1980. But that was a poor position, and Chambon feels that foreign laboratories — say in the United States with various sources of finance to call on — may have done better.

So after two years of rhetoric in support of research in France, one of France's best laboratories has had a budget increase of only 5–8 per cent. **Robert Walgate**

Hungarian science

Budget cuts weathered

HUNGARY'S scientific community must develop greater mobility, Dr Yanos Szentagothai, president of the Hungarian Academy of Sciences, said on Budapest radio recently. He was reporting on this month's general meeting of the academy, the first since last summer's traumatic cutbacks in science spending, when many researchers found themselves deprived of promised funds. The cuts were introduced so suddenly that scientists had no chance to suggest where economies might best be made, with the result that essentials were sacrificed and less important items in the science budget conserved.

During the past few months, however, the scientific community has been able to make its case to the government and the cuts have been adjusted to fall where they are least regretted. Even so, this process has inevitably generated what Dr Szentagothai described as "fear and unease".

The immediate cause of the cutbacks was the world recession, aggravated by a number of internal problems, in particular, the expansion of the Academy of Sciences. During the past thirty years, the academy has developed from a purely honorific body with no research base into a major complex of institutes and research groups, with much autonomy in the allocation of research funds and considerable fringe benefits. The academy research structure, says Dr Szentagothai, has now reached saturation point, and if young and talented graduates are to be given their chance, room must be made for them at the expense of experienced but not particularly creative senior staff.

Early retirement, the obvious answer in many countries, is unacceptable. Hungary encourages pensioners to stay on at work as long as possible. Some of the older scientists, Dr Szentagothai suggests, could well take up university lectureships.

Industry also offers considerable scope. There are at present a number of research areas designated for priority resource allocations — pharmaceuticals, aluminium metallurgy, petrochemicals and protein production, for example — which industry is committed to support. Yet industrial managers are not eager to establish their own research facilities, preferring to contract out research projects to scientists already working in the universities or academy institutes.

Dr Szentagothai's suggestion, that the prestige of research work within the industrial sector should be raised and that older scientists should make way for newcomers would therefore appear to depend on persuading industrial managers to provide research jobs and facilities within their factories. **Vera Rich**

Lopsided Z^0 particle tracked down?

DR Carlo Rubbia of Harvard University revealed in a talk at Princeton University last week that his group at CERN, the European Organization for Nuclear Research in Geneva, may have detected the Z^0 particle, the third of the intermediate vector bosons predicted by theory. Rubbia's group detected the two other particles, the W^+ and W^- , last January.

Rubbia revealed the finding in a general talk on his group's research. "He showed one event that's spectacular", said Dr Pierre Piroué of Princeton, who attended the talk. "There's no question it could be a Z^0 ". But one event is not considered enough to justify an announcement that the particle has definitely been detected.

Rubbia's evidence is the detection of an electron and a positron, the predicted decay particles of the Z^0 . The event occurred close to 100 GeV, which is the predicted mass of the new particle. According to Piroué, the exact mass implied by Rubbia's observation was actually slightly higher than that predicted by theory.

Rubbia and his group are resisting the temptation to make a public announcement, but an "internal statement" is circulating at CERN. The statement is said to

have been written by Rubbia and is headed "First sign of the Z^0 ?" (the question mark is significant). It reads:

"During the night of 30 April a remarkable event was recorded by the big UA1 detector. Two very high energy tracks were recorded, and identified as an electron and a positron from their calorimetry, flying away from each other at a wide angle. The most likely interpretation is that they come from the decay of the Z^0 particle."

A CERN spokeswoman said that Rubbia would not wish to make an announcement until he has 4 or 5 similar events. But there may be a stronger reason for caution.

It is widely accepted that the Z^0 should be recognizable by its decay into an electron and a positron. In Rubbia's putative event, however, the electron track has an energy of 50 GeV but the positron is said to have only 8 GeV, the rest of the 50 GeV on that side of the event being found as radiation. Although positrons (and electrons) radiate strongly on passing through matter, the conversion of such a high proportion of the energy into radiation is unusual. To that extent the event is an oddity, and so not one on which to stake a reputation.

Stephen Budsonsky & Robert Walgate

British election campaign

Education a contentious issue

SWEEPING changes in the administration of British higher education have been promised by two of the three main political parties fighting the general election on 9 June. Both the Labour Party and the Alliance of the Social Democratic Party (SDP) and the Liberal Party advocate increased access to higher education. The Conservative Party manifesto was not due to be published until Wednesday of this week, but must be supposed to be at least defensive of the government's policy in the past four years.

The Alliance manifesto promises to increase access to higher and further education and to establish a new Ministry of Education and Training that would combine the functions of the Manpower Services Commission and the education departments. The Alliance also promises to review courses to ensure that industry is provided with suitably qualified people even if students have to take a wider range of courses before moving on to a job or more specialist education.

SDP's policy document on education and training expands on the theme, suggesting a shift from single-subject degrees to broadly-based two-year general degrees in which all undergraduates would study both arts and science subjects. This would be followed either by a two-year vocational qualification with "on the job" training or by a further academic qualification.



SDP's document (with which the Liberals will largely agree) also questions whether it is necessary to sustain the dividing line between universities and polytechnics and whether a new body should succeed the University Grants Committee (UGC) and the National Advisory Board (NAB) to distribute funds.

On science and technology, SDP wants funds to be more selectively applied and concentrated more heavily on strategic and applied research; this, it says, will require an explicit national science policy. Generous incentives should be given to companies commissioning research and development from such institutions. The Alliance manifesto promises increased government assistance to private industry, to encourage modernization and to stimulate innovation, with a corresponding reduction in defence research and development.

The Labour Party manifesto calls for "expansion with change" in post-18 education. It promises to give priority to adults denied educational opportunity, and to increase the supply of qualified engineers and technicians.

Labour's discussion document, "Education with Change", calls for a new general principle: that "all full time and part time post-school education should be available to all those who wish to take up approved courses designed to meet their needs, regardless of age". The party favours the abolition of fees for most students. Entry into higher education would be by a modular credit system that would give qualifications and learning experience appropriate recognition. UGC would be replaced by a new, more open body, the document says. This would be a

two-tier system with representatives from unions, local authorities and the Department of Education and Science on the one hand and from the universities on the other. The party intends eventually to end the distinction between universities and polytechnics.

On university research, the party endorses the present dual system of support, but says adequate levels of support need to be restored. It wants frequent reviews to ensure a proper balance between applied and fundamental research and a peer review system to monitor research output.

Labour has not produced a detailed science and technology document, but its study group's interim report says that Labour will reduce the proportion of gross domestic product spent on defence-related research. In order to help industry, Labour says it will form a new National Investment Bank to channel funds from financial institutions into long-term investments in new technology.

Tim Beardsley

UK higher education

Royal Society warning on cuts

THE Royal Society has fired an effective shot in the battle about British higher education, with a calculation, published last week, of the extent to which social mobility will affect the demand for higher education in the two decades ahead. Although the falling number of 18-year-olds throughout the 1980s expected on demographic grounds has been used partly to justify reduced provision of higher education, the Royal Society concludes that the number of would-be students will be constant. And although the size of the 18-year-old age group will fall by 27 per cent between 1989 and 1995, the demand for higher education is likely to decrease by between 17.5 and 19 per cent.

The calculation has been produced by the Royal Society's "committee on university funding", but is largely the work of Dr P.M.D. Collins of the society's staff. The document now published says that attempts to predict future demand for higher education from gross birthrate trends are "seriously misleading", and that the planned sharp contraction of provision for higher education could deny educational opportunity to those qualified to benefit.

Two factors underlie the calculation — the proportionately greater demand for higher education among the children of the higher social classes and the slow but apparent upward social mobility of the British population. Using the pre-1981 definitions of social class, the proportion of the children whose parents are in social classes I and II (professional and managerial respectively) applying for higher education places increased from 60 per cent in 1977 to 64.6 per cent in 1981. Estimates of social mobility have been constructed from a Labour Force Survey carried out in 1981 which provided data about

the distribution of the 15-year-old population among households of different social class. On this basis, the calculation infers that the proportions of 18-year-olds in social classes I and II are increased by 1.5 per cent and 9.0 per cent by recruitment from other social classes.

Assuming no change in the proportion of 18-year-olds applying for places in higher education, the Royal Society estimates that the annual demand for student places will fluctuate narrowly about 155,000 until 1989, will decline sharply by about 30,000 between then and 1995 but will thereafter increase sharply again as the effects of the increased birthrate in the past few years become apparent. □

Credit where it's due

"... WE launched Information Technology. Now we are trying to help the manufacturers of new chemical products. In Britain we are particularly good with pharmaceuticals. Cloning was a British discovery — we got a Nobel Prize for it — César Milstein down at the Molecular Biology lab in Cambridge..."

So said Margaret Thatcher, the British Prime Minister, on her government's sponsorship of industrial innovation, in an interview published in *The Observer* on 8 May. In fact S. Cohen (Stanford University) and H. Boyer (University of California at San Francisco), the holders of the basic (but disputed) cloning patent have not yet been summoned to Stockholm. And Dr Milstein, an Argentinian by origin now at the MRC Laboratory of Molecular Biology at Cambridge, has not yet been awarded a Nobel Prize for his work on monoclonal antibodies. □

True identity of a diffraction pattern attributed to valyl tRNA

SIR — We have examined in detail several publications by H.H. Paradies. One is a report in *Nature* on 11 April 1970 about single crystals of a valine-specific tRNA from yeast¹. We find that the diffraction pattern attributed to valyl tRNA in Fig. 2 of that article is in fact an X-ray photograph from a crystal of human carbonic anhydrase B. The circumstances are such that we see no alternative but to conclude that this was a deliberate misrepresentation.

Even a rather superficial reading of the paper raises suspicions.

First, there are implausibilities. The crystal that produces Figs 1 and 2 diffracts exceptionally well for a macromolecular crystal of the small size reported even in comparison with the best tRNA crystals yet characterized. That such crystals should suddenly be "completely destroyed" after short X-ray exposure is not plausible. It is also unbelievable that Fig. 3 does not show a beam-stop shadow.

Second, there are internal inconsistencies. Contrary to the statement that "proper alignment . . . could not be achieved", Figs 1 and 2 are aligned to within 1° and 0.3° respectively. If the precession angle for Fig. 2 were 3.5°, as stated, then one would expect from the reported cell constants of 94 and 80 Å that respectively 7 and 6 orders of diffraction should appear in the inner circle of the pattern with CuK α radiation. Instead, there are only 5 orders in each direction and the ratio of the spacings in this net is actually 1.09±0.01, not 1.18 as for the reported parameters. Also, this diffraction pattern shows *mm* symmetry, even in upper levels; thus the angle γ must be 90° exactly not approximately so as stated.

If the published account cannot be believed, then what is the true identity of the crystal that produced Fig. 2? In principle, the lattice parameters of a crystal can be determined from a well aligned screenless small-angle precession photograph such as this. Normally one would know the precession angle μ and the effective magnification factor F (the product of crystal-to-film distance and photographic enlargement), but in this case these too are unknowns.

Fortunately, these parameters as well as the interplanar spacing d that is normal to the aligned nets can be determined from the bounds of upper levels in the photograph. It can be shown that diffraction from the n th layer occurs on the film in an annulus bounded by limiting radii of

$$R_n(\text{outer}) = F[\cos\mu \tan[\cos^{-1}(\cos\mu - \frac{n\lambda}{d})] \pm \sin\mu] \quad (1)$$

where λ is the X-ray wavelength. Lattice dimensions within the aligned nets can be obtained from the spacings X_n in the n th annulus on the film according to

$$a = \frac{F\lambda}{(1 - n\lambda/d)} \cdot \frac{1}{X_n} \quad (2)$$

These formulae assume perfect alignment. However, misalignment error of the magnitude present in Fig. 2 of ref. 1 causes negligible perturbations. In this case, all unit cell angles must be 90° since the pattern shows *mm* symmetry throughout.

Lorentz factor enhancement at the bounding edges of diffraction annuli in the precession geometry makes the limiting radii in a diffraction pattern readily measurable. These were at 8.1, 23.2, 31.4, 36.1, 44.0 and 46.5 mm respectively for $R_0^{\text{outer}}, R_1^{\text{inner}}, \dots, R_5^{\text{inner}}$ in a photocopy of Fig. 2 from the journal page. A least-squares fit of equation 1 to these data gave $F = 89 \pm 4$ mm, $d = 35 \pm 3$ Å and $\mu = 2.6 \pm 0.1^\circ$ with an r.m.s. residual of 0.12 mm. When constrained to $\mu = 2.5^\circ$, the nearest likely setting in common laboratory practice, the fit produced $F = 92.1 \pm 1.2$ mm and $d = 37.8 \pm 0.8$ Å with an r.m.s. residual of 0.14 mm. Spacings in the first annulus were $X_1 = 1.986 \pm 0.007$ mm vertically and $Y_1 = 1.882 \pm 0.006$ mm horizontally. Then, by equation 2, these values together with parameters from the $\mu = 2.5^\circ$ fit of equation 1 to the radii measurements gave lattice constants of $a = 74.4 \pm 1.0$, $b = 81.4 \pm 1.1$ and $c = 37.8 \pm 0.8$ Å. As the cell is clearly primitive and orthorhombic and we assume that handed biological molecules were crystallized, the space group is necessarily either P2₁2₁2₁, P222 or a permutation of P2₁2₁2 or P222₁.

When tentative crystallographic parameters had been determined we communicated them (without other comment) to Dr Gary Gilliland at the National Institutes of Health, whom we knew to be compiling a library of crystal data on macromolecules, and asked that he search for any crystal having these dimensions and symmetry. Only human carbonic anhydrase B (HCAB) of the nearly 700 entries in his data base at that time came close to having these dimensions. HCAB crystallizes in space group P2₁2₁2₁ and its reported cell constants are $a = 81.5$, $b = 73.6$ and $c = 37.1$ Å (refs 2,3). At this point, Fig. 2 was checked against extant X-ray photographs of HCAB in Uppsala. Although such a screenless precession photograph could not be found, the relative diffraction intensities in (hk0), (hk1) and hk2) nets were seen to agree well with the published pattern. This leads us to the unambiguous conclusion that Fig. 2 of ref. 1 is an X-ray photograph of an HCAB crystal.

We have considered the possibility that the crystals were actually grown from a sample of carbonic anhydrase that had inadvertently been mistaken for one of tRNA. Paradies certainly had access to HCAB when he worked on tRNA in the Wallenberg Laboratory. However, such a mistake could not be the fault since the

reported conditions for crystallization differ markedly from those needed for HCAB. HCAB crystals grow from a 2.3M ammonium sulphate solution at pH 8.7 (ref. 2) whereas the valyl tRNA crystals are said to grow from 20 per cent solution of dioxane-water at pH 7.5 (ref. 1).

We also reject the hypothesis that HCAB photographs were accidentally inserted in the place of true tRNA patterns. It seems unacceptably implausible that such a mistake could occur and then remain undetected when Paradies proof-read and subsequently cited the article. Thus we are forced to conclude that the misrepresentation was intentional. The coauthor of the paper in question was not involved in this deception and did not see the manuscript before its publication. His role in the study was limited to supplying purified tRNA. He is not a crystallographer and had no reason to believe that Fig. 2 was wrong.

This is not an isolated incident. We have extensive documentation of similar misrepresentations in other articles published by Paradies. In one⁴, diffraction patterns from crystals of human carbonic anhydrase C (HCAC) are falsely represented as being from seryl tRNA crystals. In another⁵, a diffraction photograph from a crystal of HCAC is falsely described as one from a crystal of valyl-tRNA synthetase from *Escherichia coli*. Two other papers ascribe the same optical transform and inverted photographic images of the same electron micrograph to very different substances. In one case⁶ they are attributed to a negatively stained thin section from a crystal of algal D-ribulose 1,5-bisphosphate carboxylase, and in the other⁷ a stained thin crystal of spinach chloroplast coupling factor. One or both of these publications must be false, but there is no reason to suspect any complicity by coauthors of ref. 6 in any misrepresentation. Still other papers^{8,9} contain such serious flaws (for example major inconsistencies between descriptions in the text and the raw data in figures) that we believe they should be discounted even though we cannot prove them fraudulent.

WAYNE A. HENDRICKSON
Laboratory for the Structure of Matter,
Naval Research Laboratory,
Washington DC, USA

BROR E. STRANDBERG
ANDERS LILJAS

Wallenberg Laboratory,
University of Uppsala, Sweden

L. MARIO AMZEL
EATON E. LATTMAN

Department of Biophysics,
Johns Hopkins University School of Medicine,
Baltimore, Maryland, USA

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A reply by Professor Paradies follows overleaf.

A reply from Paradies

SIR — My 1970 paper is not a misrepresentation because:

(1) The crystal diffracts well and does not deteriorate as long as the temperature is below 4°C. But within 4 h of increasing the temperature from 4°C to 21°C the vapour pressure of *t*-butanol/dioxane increases more than 10-fold so that the tRNA crystal dissolves. Therefore, the loss of the high angle reflections in Fig. 3 after 3 h of X-ray exposure at 21°C (the crystal was actually at 21°C for more than 4 h) is not unexpected. An additional cause of crystal destruction is damage by X-ray-induced tertiary butanol radicals and short-lived dioxane radicals. The statements by Hendrickson *et al.* of the "short X-ray exposure" and the beam stop shadow and the expression "suddenly be completely destroyed", are unfounded. Even for obtaining the X-ray picture, shown in Fig. 2, 90 minutes were necessary, as stated.

(2) The tRNA crystal was definitely not a small one; for obtaining the general cell dimensions, a crystal size of 0.1 mm would be sufficient for a macromolecule with a molecular weight of 25,000. Since the intensity is roughly proportional to the diffraction volume of the crystal, the reported crystal size of $0.2 \times 0.15 \times 0.2$ mm yields a diffracting volume six times that needed for getting the cell dimensions and, if it is stable at 21°C, has almost the quality, from the point of intensity distribution of the spots and diffracting volume, for high resolution X-ray analysis.

(3) Hendrickson *et al.* recalculate from Fig. 2 of ref. 1 the ratio of the spacings in the nets as 1.09 but that is meaningless because the magnification, not indicated in Fig. 2, is different in the horizontal and vertical planes. In the horizontal plane it is $\times 1.45$ – 1.48 and in the vertical plane it is $\times 1.35$ – 1.37 , revealing a ratio of about 1.085–1.09. Multiplication of the ratio of the net spacings of the X-ray pattern of Fig. 2 with the ratios of the enlargement, 1.09×1.085 , yields 1.18 (ref. 3), which is consistent with the reported parameters. Since the film-to-specimen distance is 75 mm, and not 60 mm, as Hendrickson *et al.* impute, the dimensions of the net are calculated to 94 Å and 80 Å in the low angle region, in the *kh1* plane to 94.05 Å and 80.21 Å, and in the *hk2* plane to approximately 94 Å and 79–80 Å. However, a calculation of the net, assuming $F = 60$ mm, leads to values of 75 ± 1.5 Å and 64.2 ± 2.0 Å, respectively. Neither value for F (75 mm or 60 mm) is consistent with those for a crystal of human carbonic anhydrase B with $a = 74.4$ Å, $b = 81.4$ Å and $c = 37.8$ Å. Spacings in the first annulus were measured to be 1.228 horizontally and 1.440 vertically. The third axis is calculated to be 34–35 Å, with $F = 75$ mm, which also is not consistent with the *c*-dimension of

carbonic anhydrase.

(4) The X-ray pattern does not show complete *mm* symmetry; this is more pronounced at higher levels, which might be due to the misalignment and slipping of the crystal, causing the perturbations that are clearly to be seen. Furthermore, the unit cell angles in the upper levels are not all 90°C. Therefore, this pattern and the intensity distribution in the *hk1* and *hk2* layers are not those of a crystal of human carbonic anhydrase B. Nor would it have yielded the observed *hk0* intensity distribution, including the unusual distribution of dots coming from the β -radiation. Although the unit cell is apparently orthorhombic and there is some indication of *mm* symmetry the possibility exists that the crystal symmetry with respect to higher resolution (see above) is monoclinic.

In conclusion: cell dimensions, crystal stability and solubility, including temperature dependence, are consistent with a crystal of tRNA.

With respect to my other papers:

(1) The net of the diffraction patterns for the seryl-tRNA crystals (2) was calculated by a colleague in Uppsala, Sweden, and it revealed almost the same result as that previously obtained from a powder pattern, which was also included in that papers.

(2) There are no inconsistencies between the measured *d*-spacings and the figure in my 1968 paper³.

(3) The crystals of valyl-tRNA synthetase⁴ are not a misrepresentation of a crystal of human carbonic anhydrase C. The crystal in Fig. 4 is vertically misaligned from the (001) axis, caused by the crystal having slipped in the capillary during exposure. The mentioned orthorhombic crystals of the synthetase are not documented, only the cell dimensions. And, again, magnifications were not stated on the figure.

(4) My contribution to the characterization of crystals of D-ribulose 1,5-diphosphate carboxylase⁵ was only the determination of the crystal density. The electron micrographs were obtained by my co-authors and the optical diffraction pattern was obtained with the facilities of Dr Räuber, Fritz-Haber-Institut, Berlin-West. Assuming a molecular weight of 515,000–550,000, all reported results in this paper are correct, including the magnitude of V_M , with no assumption made of the number stoichiometry of the large and small subunits.

(5) The very unfortunate mistake by which the electron micrograph and its optical diffraction patterns of ref. 5 were used again in my paper on spinach chloroplast coupling factor⁶ was corrected as soon as the error was brought

to my attention⁷. The results in both my 1979 paper⁶ and my 1980 paper⁸ were compared with other physical measurements (see also ref. 6).

H.H. PARADIES

D-1000 Berlin 45,
FRG

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Evolution by numbers

SIR — In the discussion in your columns about the application of quantitative methodology based on the study of evolutionary processes to the analysis of the development of human culture^{1,2}, there is an unquestioned assumption on both sides of that issue that quantitative theory, as expounded by practitioners such as Fisher, Haldane, Wright, Cavalli-Sforza and Maynard Smith, has been successful in illuminating and explaining the process of biological evolution and the genetic relationships between species. As far as I know, there is no evidence to support this assumption. Indeed, there is a vast number of observations unaccounted for in the extant quantitative evolutionary theories. Many of these observations (inducible mutation systems³, rapid genomic changes involving mobile genetic elements⁴, programmed changes in chromosome structure^{5–8}) challenge the most fundamental assumptions which these evolutionary theories make about the mechanisms of hereditary variation and the fixation of genetic differences.

As a practising geneticist, I am frequently astonished by the ease with which population theorists assume complex (and therefore troublesome) phenomena out of existence, no matter how solid the documentation. Perhaps they should set their own house in order and come to terms with what genetics and molecular biology have to teach them about possible mechanisms of biological evolution before they try to save anthropology from the anthropologists.

JAMES A. SHAPIRO

Department of Microbiology
University of Chicago, USA

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Disputed X-ray data unresolved

The letters from Hendrickson and Paradies on the two preceding pages raise doubts about the authenticity of a series of published papers. Here is a guide for readers.

THE resignation as of mid-April 1983 of Professor Hasko Paradies from his chair of biology at the Free University of Berlin comes a year after the university had started an investigation into charges of scientific fraud. Paradies leaves behind him a string of disputed publications and the question of how he survived so long in a career that was first challenged in 1970.

The university's investigation was begun on the basis of a dossier compiled by Dr Wayne Hendrickson of the Naval Research Laboratory in Washington DC, whose doubts about some of Paradies's published work are expressed on page 195 followed by a reply from Paradies. If true, the charges are dismaying.

It was a seminar given by Paradies in March 1982 at the Naval Research Laboratory before he spent a sabbatical there that spurred Hendrickson into action. But he was already aware of doubts about Paradies stemming from his departure from King's College in 1970.

Paradies had been given space at King's College, London, to determine the crystallographic structure of transfer RNA, the family of molecules that carry specific amino acids to their site of assembly into proteins according to the instructions of the genetic code.

Crystallographic structure determination cannot begin without a crystal and Paradies first came to prominence with his claim, at a meeting in 1967, to have produced crystals of transfer RNA. It also brought him into contact with the Medical Research Council's Laboratory of Molecular Biology in Cambridge, where Brian F. C. Clark was also trying to crystallize transfer RNA and from which Kenneth Holmes was about to join the Max Planck Institute for Medical Research in Heidelberg.

Holmes offered Paradies, then aged 26 and with an MD from the University of Munster, a job. He was first to go to the University of Uppsala to learn about crystallography from Bror Strandberg and then to come to Heidelberg. It was from Uppsala that Paradies's first paper on the crystallization of transfer RNA appeared in 1968. It was also from Uppsala that Paradies visited Brian Clark's laboratory, which was finding difficulty in reproducing Paradies's protocol for the crystallization.

Paradies arrived without his own crystals, claiming they had been lost in an accident on the ferry from Germany to Sweden and, according to Clark, failed to produce any more crystals in his month or

so at Cambridge. Shortly thereafter Clark succeeded in getting crystals by a modification of the Paradies protocol. Clark published his results in *Nature* (219, 1222; 1968) and generously, it may be thought, made Paradies a co-author on the paper for having stimulated the protocol that was eventually successful.

Paradies continued his studies at Uppsala from where (but some time after he had left) he published the first diffraction pattern from a transfer RNA crystal (the 1970 *Nature* paper that is the focus of Hendrickson's accusation of "deliberate misrepresentation").

From Uppsala, Paradies went to Heidelberg, as planned, but stayed there only a few months, to appear at King's College London asking for space to pursue his work. Watson Fuller recalls that Paradies came at the end of 1969 with good qualifications and a reputation as immensely energetic and somewhat eccentric.

By September 1970, Paradies was claiming in King's College to have more good crystals of transfer RNA and excellent X-ray diffraction patterns from them. By chance David Blow paid a visit to the King's College laboratory and had a chance to look at Paradies's diffraction patterns. Blow was certain there was something odd about what he was shown and asked Paradies some questions that led him to produce other prints from his files. It was then that Blow recognized one of the prints as from his own crystallographic studies of chymotrypsin. Blow recalls that Paradies admitted as much when challenged. Paradies says that it was Watson Fuller, not Blow, who raised the issue of chymotrypsin.

After that incident, Paradies was advised to leave King's College. He returned to his native Germany but was back in October saying that he had been offered a job by Heinz-Gunter Wittman in his Max Planck Institute in Berlin. That news led to a letter from Watson Fuller to Wittman in December 1970 in which the full circumstances of Paradies's departure from King's College were explained.

Nevertheless, Paradies got his job with Wittman and there he stayed until 1974 when he obtained his tenured post of professor of biology at the Free University of Berlin. Dr H. Ibbeken, now vice-president of the university, who was in charge of the investigation earlier this year, says Paradies's appointment followed regular procedures, which would have included

obtaining Wittman's opinion.

Paradies published one paper from Wittman's laboratory on crystallized ribosomes — and three from his university claiming the crystallization of important enzymes. It is Hendrickson's contention that all of these papers are dubious and that the earlier papers on transfer RNA crystals are fraudulent.

His suspicions aroused early last year, Hendrickson carefully examined all of Paradies's publications. As detailed on page 195, he decided to determine the parameters of the crystal whose X-ray diffraction pattern was published in Paradies's 1970 paper in *Nature*. When Dr Gary Gilliland was asked, without being told why, to match the calculated parameters to those of proteins in a library of data he was compiling, he came back with the answer — carbonic anhydrase B, one of the proteins studied at Uppsala when Paradies was there.

Paradies, in his reply on page 196, denies the charges. The crux of his reply is that Hendrickson's recalculation of the crystal parameters is meaningless because "the magnification, not indicated in Fig. 2, is different in the horizontal and vertical planes". That, says Hendrickson, is not altogether impossible but is very hard to believe. David Blow goes further. "Paradies's explanation is not credible", he says, "because the curves which appear as parts of circles in the figure would have come out as ellipses" had there been different magnifications.

Presumably the Free University of Berlin had come to similarly trending conclusions when its investigations led it early this year to seek to remove Paradies from his post. That entailed ministerial approval which eventually was forthcoming. Shortly afterwards Paradies resigned but, he said this week, for reasons completely unconnected with the investigations. The university's letter accepting the resignation makes no mention of the investigation, he added.

In his wake are a cluster of disputed publications analysed by Dr Hendrickson and another cluster, some of which arise from periods when Paradies visited Cornell University from Berlin, that will no doubt now be scrutinized. But there remains some degree of praise from all those involved in early attempts to crystallize transfer RNA. Whether or not Paradies ever himself produced such crystals, they say, his protocol for doing so included at least one innovative step.

Peter Newmark

Plant genetic engineering

Launching genes across phylogenetic barriers

from Martin Drummond

THE extraordinary strategy for survival evolved by the Ti plasmids of the *Agrobacteria* depends on the transfer of a segment of plasmid DNA from the bacterium into the nuclear genome of a higher plant. There the transferred segment, T-DNA, directs the synthesis of low-molecular-weight metabolites termed opines which are absent from normal plant tissue. The Ti plasmid also encodes a permease and oxidase for degrading opines, which thus serve as a carbon and nitrogen source for the host bacterium. Furthermore, some opines derepress plasmid transfer functions so that in the presence of its substrate the catabolic plasmid can spread rapidly through a mixed population of bacteria. By diverting the biosynthetic resources of the plant the Ti plasmid generates selective pressure for itself; and that pressure is further amplified by a more conspicuous effect of T-DNA — that of causing plant cells to divide in an uncontrolled way to form a crown gall tumour or teratoma.

As soon as Chilton *et al.*¹ demonstrated unequivocally the presence of T-DNA in crown gall tissue the possibility of using Ti plasmids as vectors for the genetic engineering of higher plants was raised. In this issue of *Nature*, Herrera-Estrella *et al.*² describe how a final obstacle to obtaining expression of foreign genes in plants has been overcome. The first problem was how to insert passenger sequences into a molecule as big as a 200-kilobase Ti plasmid. This difficulty was neatly side-stepped by using standard *in vitro* methods to insert passenger sequences into T-DNA cloned on a small plasmid, and then using recombination *in vivo* to get the modified T-DNA back into the Ti plasmid³.

A number of foreign genes have now been introduced into higher plant cells, including a bacterial gene for dihydrofolate reductase, a mammalian gene for interferon, and that encoding the bean storage protein phaseolin. Hitherto there have been no confirmed reports of efficient expression of any foreign protein in crown gall tissue, although transcription of the phaseolin gene could be detected⁴, given the differences between promoter sequences in prokaryotes and eukaryotes, it is not surprising that bacterial genes do not generally function in plant cells. Nor is it remarkable that mammalian genes or developmentally regulated plant genes from another family fail to be expressed in tumour tissue cultured *in vitro*. Herrera-Estrella *et al.* have solved part of this problem by fusing foreign genes to a T-DNA promoter which normally directs expression of a synthase catalyzing opine production.

Ti plasmids may be classified according to the opine they determine, two intensively studied classes being octopine and nopaline plasmids. Recently the genes for octopine synthase, *ocs*, and nopaline synthase, *nos*, have been sequenced, and it seems that although they originate from a bacterial cell they are preceded by typical eukaryotic promoters and have what appear to be eukaryotic polyadenylation signals near the 3' end. Herrera-Estrella *et al.* have constructed an 'expression' vector, pLGV2381, which carries the *nos* promoter and nearly all of the leader transcript sequence separated by a single *Bam*HI site from the 3' end of *nos* with its polyadenylation site. Thus any coding sequence inserted at the *Bam*HI site will be expressed under the control of *nos* regulatory sequences. To demonstrate the efficacy of this vector they first inserted the coding sequence of octopine synthase without its promoter (the *ocs* cassette) and then the bacterial gene for chloramphenicol acetyltransferase. Both are transcribed and translated when transferred to the tobacco genome via a nopaline Ti plasmid, octopine synthase somewhat more strongly than when under the control of its own promoter.

These products are synthesized in tumour tissue, but to be of practical importance fully differentiated plants must be recovered from engineered tissue. The separation into functional groups of the plasmid genes involved in crown gall makes this possible. The 'virulence' genes all lie outside the T-DNA and seem to be responsible for DNA transfer to the plant, whereas the 'oncogenic' genes map within T-DNA and cause tumorous growth by disturbing growth hormone levels in the plant cell⁵. It is sufficient to delete part or all of the oncogenic group of genes to 'disarm' the Ti plasmid so that it transfers what remains of the T-DNA but does not prevent the recipient plant cell from redifferentiating after growth in tissue culture⁶. Using this approach Chilton's group has produced fully differentiated tobacco plants in which yeast alcohol dehydrogenase is expressed under the control of the *nos* promoter.

Hitherto it has been difficult to dispense with the oncogenic genes since the hormone independence they confer provides a convenient way of selecting transformed cells in tissue culture. Now, however, it should be possible to place a selectable marker such as resistance to the kanamycin analogue G418 under the control of a synthase promoter while replacing the oncogenic genes with the genetic package of choice.

For plant breeding it is clearly desirable

that foreign genes be transmitted through the germ line. Early fears that T-DNA was completely eliminated at meiosis turned out to be groundless, *ocs* being inherited in a satisfyingly mendelian manner⁷.

In principle the genetic resources of the entire biosphere are now available for crop improvement, but the new technology is unlikely to have an immediate impact on plant breeding because the molecular basis of many desirable genetic traits is as yet unknown, and is often determined by multiple unlinked genes. There is thus a dearth of really attractive applications. One possibility is introducing bacterial genes encoding enzymes for herbicide degradation; another is 'upgrading' plant storage proteins for animal consumption by introducing genes mutagenized *in vitro* to include more lysine codons.

Further applications depend on the development of plant molecular genetics, and here too Ti plasmids seem bound to play an important part. To examine the intergeneric conservation of developmentally regulated promoters, for example, the *ocs* cassette might be placed under the control of the phaseolin regulatory sequences and the rapid sensitive octopine assay used to determine whether this promoter is expressed in peas or sunflower seeds.

Such uses will certainly not see an end to the study of crown gall in its own right. Many basic questions remain unanswered about the mechanism by which the Ti plasmid conveys T-DNA across what one would have supposed to be insuperable genetic barriers between kingdoms. Which virulence genes, if any, determine recognition of the plant cell surface? Does the entire plasmid enter the plant cell? Are plasmid genes or plant genes or both required for the transposition event? One clue to the mechanism of transposition is the presence of 25-base-pair direct repeats flanking T-DNA in both plant and plasmid⁸, but plasmid deletions removing one border still give tumours. Plant protoplasts cultured *in vitro* can now be transformed by *Agrobacterium* and with higher efficiencies of transformation the early events in oncogenesis should become amenable to investigation⁹.

Crown gall also provides an experimental system to analyse the synthesis and regulation of plant growth hormones. Akiyoshi *et al.*⁵ have shown that mutating any one of the three oncogenic loci conserved on octopine and nopaline Ti plasmids alters the ratio of auxins to cytokinins in the tumour tissue, producing

Martin Drummond is at the ARC Unit of Nitrogen Fixation, University of Sussex, Brighton BN1 9RQ.

either roots or shoots in a manner consonant with what we know about the effect of these hormones on normal tissue cultured *in vitro*. The products of these genes could either themselves catalyze some step in hormone synthesis, or derange control of the plant's own synthetic or degradative pathways. These possibilities can now probably be investigated.

The answers to these fundamental questions will probably have profound implications for the use of Ti plasmids as vectors. Important candidates for improvement are the grass crops, but monocotyledonous plants lie outside the normal host range of *Agrobacterium*, which includes most dicotyledons and some gymnosperms. This could be because Ti plasmid DNA cannot enter the cell, or because it fails to be integrated into plant DNA, or simply because the morphogenetic restraints on cell division are not disrupted by the oncogenic genes in monocotyledons as they are in dicotyledons.

The ironic possibility that Ti plasmids are already 'disarmed' with respect to grass crops causes one to wonder if their strategem of inserting DNA into another organism is unique. It has only been

detected in the case of the Ti plasmids because of its oncogenic effect, which is not an essential part of the strategem. And is this bizarre transfer of genetic material unidirectional, or could the Ti plasmid have picked up its T-DNA sequences from a plant in the first place?

Ten years ago work on crown gall was the poor relation of cancer research. Now as a vector for crop improvement and as a research tool, as a system for studying the biochemical genetics of hormone action, and as an extraordinary phenomenon in its own right it holds a central place in the new discipline of plant molecular biology. Those who study it enjoy a rare opportunity to do basic research with immediate and far-reaching practical implications. □

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High-energy physics

Structure in electrons?

from Frank Close

ARE electrons and quarks the ultimate frontier of matter or are they perhaps built from more fundamental particles so far undetected? What hope is there of answering this question in the near future? In a recent paper (*Phys. Rev. Lett.* **50**, 811; 1983), Eichten, Lane and Peskin point out that prospects are perhaps more exciting than has been generally realized.

When we say that electrons (or quarks) are pointlike, we implicitly add the caveat "subject to the limits of resolution currently available". A plane appears pointlike to a radar beam, as does an atom to visible light. As wavelength of the probe decreases, resolution improves and new structure appears.

The uncertainty principle places an inherent limit on resolution, of course. To resolve submicroscopic distances requires high-momentum high-energy probes. This is the domain of the high-energy or particle physicist. As the power of particle accelerators has increased so have the resolvable distances been reduced. The pointlike atom of the late nineteenth century became a structure with a pointlike nucleus; by the 1950s nuclear structures were resolved; and by 1970 the proton itself was seen to contain quarks. At the best resolution now available — distances of order 10^{-18} m can be probed — no inner structure has been detected for quarks or for electrons and their associates. For all practical purposes they are structureless.

Even so, there are some tantalizing problems. Long before atomic structure was discerned, Mendeleev drew up his periodic table of the elements. With hindsight we can see here the first hint of the 'non-elementarity' of atoms. More recently the eightfold-way patterns that were noticed in the subnuclear particles (proton, neutron, pion and so on) gave the clue that deeper structure might be responsible for this regularity. And indeed it was; a decade later the quarks were seen within the 'elementary' particles.

Today we know six particles like the electron ('leptons') and five (probably six) quarks. They appear elementary to the experimentalist — just as protons and atoms once did. But there are intriguing regularities in their behaviour. 'Generation' patterns have been discerned (*Nature* **271**, 406; 1978), and even the families of quarks and those of the leptons seem to be related.

It is possible that this is a profound and direct consequence of grand unified theories of matter and forces; on the other hand it might be a consequence of a deeper structure that is common to quarks and leptons. Some of the versions of grand unified theories require substructure for some of the particles. Theorists estimate that substructure and/or new phenomena could occur at energies of the order of 1–10 TeV (= 1,000 GeV), slightly above present accelerator energies, and might already

have been manifested in some cosmic ray anomalies.

What can we say quantitatively about substructure?

If electrons contain constituents, these will manifest themselves in scattering experiments between electrons and positrons. As the collision energy approaches that corresponding to the size of the electron, Λ , the scattering cross-section will deviate significantly from the standard value predicted by quantum electrodynamics (QED) for an elementary electron.

Tests for substructure can be made by comparing data with QED predictions. QED is so well established that these tests are referred to as 'model-independent'. Eichten, Lane and Peskin (*Phys. Rev. Lett.* **51**, 811; 1983) have now shown that we can improve upon these if we are prepared to make model-dependent assumptions.

Without commitment to any specific model of substructure, they note that some general features are rather well accepted. In particular, the electron and positron, being composite, can interact by exchanging their constituents (analogous to covalent atomic forces). Eichten and his colleagues argue that the best way to search for electron substructure is to study the angular distribution of the scattered electrons and they show that if $\Lambda \sim 700$ GeV, even today's 'low-energy' experiments at PETRA Hamburg would yield a 5 per cent deviation in the angular distribution of the scattered particles relative to the standard expectations. Such an effect is not seen. To convert this into a limit on Λ requires knowledge of the nature of the constituent's interactions.

The authors consider a general class of models where the constituents have spin $\frac{1}{2}$. There are two ways in which they can interact: like standard electromagnetic interactions — 'vector interaction', or like neutrinos with a handedness — 'left handed' or 'right handed'. The forms of the angular distortions are different for the two types of interaction and the limit on Λ is stronger in the former case ($>1,500$ GeV compared with >750 GeV for the latter).

These results are interesting because some theorists believe, for quite different reasons, that a new physical scale Λ could occur between 1 and 10 TeV. An electron-positron facility that could copiously produce the Z^0 at 90 GeV should set limits for $\Lambda > 5$ TeV (the event rate at Z^0 resonance is high, so good statistics can be readily obtained). The coming generation of multi-TeV colliders should be able to detect substructure up to $\Lambda \approx 10$ –50 TeV. One may indeed hope for significant effects at energies well below Λ . If $\Lambda \approx 1$ –5 TeV then deviations from the standard model will soon be observable. If the W and Z bosons are confirmed this year at CERN (*Nature* **302**, 478; 1983) then these ideas will begin to loom larger in everyone's mind. □

Frank Close is at the Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX.

Cosmology

Did the tail wag the cosmic dog?

from Joseph Silk

ONE of the problems that preoccupy cosmologists concerns the distribution of matter over the large scales of size we see in the Universe today. Any theory of the origin and evolution of the Universe should incorporate processes leading to the hierarchy of galaxies and clusters of galaxies that have formed in the course of cosmic time. Within the big-bang theory that is now central to most cosmologists' way of thinking, neither of the two leading theories of galaxy formation from fluctuations in the primordial matter seems fully to satisfy the observations. A new theory, recently published, proposes that primeval fluctuations on a much smaller scale than galaxies may provoke the growth of much larger-scale fluctuations. The formation of such seeds could provide the key to understanding galaxy formation.

Understanding the large-scale structure of the Universe has proved surprisingly difficult. We see galaxies, typically of 10^{10} – 10^{11} solar masses, groups of galaxies, and great clusters and superclusters of galaxies, on scales of up to some tens of megaparsecs, as well as many dwarf galaxies, extending in mass down to 10^6 solar masses, but which can only be seen easily in the vicinity of our own Galaxy. This diverse array of structure is what cosmologists have tried to account for through the evolution of density fluctuations in an initially homogeneous, or nearly so, big bang. The theory has met with mixed success. Two natural scales emerge, but neither corresponds to that of a galaxy like our own Milky Way galaxy.

The most popular version starts with adiabatic fluctuations — fluctuations whose entropy per unit mass is everywhere the same and equal to that of the Universe itself. Recent theories of particle creation in the initial instants of the big bang result in a universal value of the entropy per baryon, which otherwise is one of the unexplained mysteries of the cosmos. The final scale that emerges from an initial, more or less arbitrary, spectrum of adiabatic density fluctuations is not that of a galaxy but that of a cluster of galaxies. All primordial structure on smaller scales was erased by the diffusive effects of the radiation in the early Universe.

The ensuing implications for galaxy formation lead to a rather convoluted series of events. The large-scale inhomogeneities from which small-scale structure, once present, has been erased by well understood physical processes, eventually collapse under the influence of gravity. These gigantic clouds fragment by way of a pancake-like collapse to compressed sheets of matter. The initial fragments are clouds of masses comparable with those of dwarf

galaxies, and these clouds rapidly coagulate to form galaxies of larger mass.

A variant on the adiabatic theory begins with isothermal fluctuations. These are entropy variations, and are not in accordance with theories of baryosynthesis and a universal entropy per baryon. However isothermal fluctuations could also be created by subsequent events, involving energy dissipation, for example, and their evolution does lead to the survival of primordial small-scale structure. The characteristic scale at the onset of galaxy formation corresponds to about one million solar masses. Above this scale, gravity dominates pressure forces once gravitational collapse can occur after the epoch of matter-radiation decoupling at a million years or so after the big bang.

Still smaller scales are possible if axions dominate the mass-density of the Universe. These hypothetical elementary particles, predicted by a certain theory of nuclear interactions, are bosons — whose phase space density, unlike that of neutrinos, is not restricted by the Pauli exclusion principle. Hence axions have recently become an interesting but hypothetical source of dark matter, since they could be dynamically important over a very wide range of scales. From the perspective of primordial fluctuations, the fact that axions are collisionless particles over much of the evolution of the Universe means that pre-existing fluctuations would not have been erased down to scales of over a solar mass.

None of this is very promising for the problem of understanding how a typical luminous galaxy has formed. If the answer is indeed that galaxies have formed by complex dissipational and dynamical processes, and any memory of primordial structure has been erased, this would inevitably disappoint many cosmologists — not all, however, as is illustrated by an interesting recent paper by Hogan (*Monthly Notices of the Royal Astronomical Society* **202**, 1101; 1983).

Hogan postulates the presence of initial seeds, perhaps of stellar mass, and shows that a not unnatural sequence of events in the early Universe may lead to the formation of secondary structures on scales up to those of the great galaxy clusters. The only truly primordial fluctuations are the stellar seeds. The release of large amounts of ionizing radiation from the seeds, which may be either massive stars or supermassive quasar-like compact objects, heats and ionizes a large volume of gas. This has the effect of generating a gradient of pressure that in turn drives large-density fluctuations. Thus, small-scale seeds have stimulated formation of large-scale irregularities. In time, the inhomogeneities

grow larger by gravitational instability and eventually form bound structures.

Two attractive features emerge naturally from Hogan's scenario. The limiting scale on which fluctuations are generated is the maximum Jeans mass in the history of the Universe, for the Jeans scale determines the maximum scale over which pressure-driven fluctuations can propagate. This scale is about 10^{16} solar masses in the conventional hot big-bang theory. Furthermore, if the seeds form by a random process, the fluctuation distribution will then be spatially uncorrelated. Now the gravitational clustering theory for formation of galaxy groups and clusters has been shown to give realistic models of the observed galaxy distribution. Implications of this theory are that the initial fluctuations were more or less random, and that the largest scales to have collapsed were of about 10^{15} or 10^{16} solar masses.

All of this agrees nicely with the theory of primordial seeds — but can the theory be tested? One implication is that there should not be any intrinsic large-scale structure in the Universe. Preliminary reports of the detection of an intrinsic quadrupole anisotropy in the cosmic background radiation appeared fatal to this theory. However between the submission of Hogan's paper and its publication, the quadrupole anisotropy disappeared: that is to say, more careful experiments failed to confirm it. These experiments set a low upper limit of about 3 parts in 10^5 on any possible quadrupole anisotropy (*Nature* **302**, 478; 1983).

Forthcoming observations of anisotropy on intermediate angular scales of a few degrees should eventually provide a sensitive test of the primordial seed theory. For if intrinsic primordial structure were present on galactic cluster scales, fluctuations in the background radiation are inevitable at a level of about one part in 10^5 .

The ultimate question in such a theory, of course, concerns the nature of Hogan's seeds. Here one runs into a fundamental difficulty. The stability of the hot big-bang model to the growth of infinitesimal perturbations has been thoroughly investigated. Certainly, at epochs where the physics is well understood, it seems to be stable. At these epochs, the horizon size, which determines the maximum scale over which causally generated fluctuations could spontaneously arise in a phase transition, contains far less than a solar mass. Hence it seems impossible to generate the required stellar mass seeds.

There are two ways to circumvent this conclusion. At much earlier epochs, close to that of the big-bang singularity itself, the horizon structure of the Universe may differ greatly from that predicted in the conventional big-bang model. Such is the case if the Universe undergoes a prolonged phase of exponential inflation, as expected in certain theories of grand unification in particle physics. Fluctuations may rise spontaneously during the transition from

an inflating universe to the conventionally expanding model, although there seems no reason to expect their scales to be limited in any way. More exotic types of phase transition have also been postulated, involving strings or walls of vacuum energy that, before eventually decaying, can generate inhomogeneities.

An alternative and even more radical approach is to dispense with the hot big bang entirely. It is the high temperature and entropy of this model that creates such difficulties for fluctuation growth. In an initially cold (or even lukewarm) big bang, spontaneous growth on stellar mass-scales

appears likely, if not inevitable, during the early transition from a solid to a gaseous phase. This model may provide the required seeds, but of course it creates other difficulties, notably the need for alternative explanations of the cosmic background radiation and of the light-element abundances, two pillars of the conventional big-bang theory. □

Joseph Silk is on sabbatical leave from the University of California, Berkeley at the Institut d'Astrophysique, 98 bis Boulevard Aragon, 75014 Paris.

Island biogeography

Variations in population density and extinction

from Mark Williamson

UNTIL recently, the biological study of plant and animal populations on islands has been focused principally on the number of species found, and population density has been largely ignored. That emphasis is now changing, and some new analyses of the population dynamics of species of spiders, lizards and birds on two groups of islands have helped to illuminate the control of population density, the probability of extinction and the detection of subtle habitat changes through changes in population density.

Standard approaches to island biology include plotting the number of species against the area of islands, as around New Zealand¹, plotting numbers of extinctions and immigrants against the number of breeding species on an island², and incidence curves³ showing the proportion of islands of a given size occupied by different species. All these are second-order results, the first-order ones being the effects of habitat density, geographical isolation and competition and predation on the population densities of the species of an island. The effect of dynamic interactions on the secondary phenomena has been particularly unclear².

Schoener and Toft⁴ show what might be done to clarify the importance of competition and predation by studying orb-web spiders on very small islands in the Bahamas. In some species of orb-web spiders, individuals can be counted on their webs. Spiders come in the middle of food chains, being predators of arthropods and prey for many vertebrates, though on the islands studied, which vary from 50 m² to less than 1 hectare, the only vertebrates are lizards (mostly *Anolis sagrei*) and then only on some of the islands.

It seems that the spiders disperse to, and maintain populations on, such small islands with difficulty. Half the islands had no spiders, and there is a marked effect of distance from larger islands: no island

more than 1.5 km from a large source island had any spiders. Allowing for this effect, islands with lizards had only one-tenth the population density of all species of spiders of islands without lizards.

Is this the result of predation by the lizards, or competition with the lizards for other arthropods? The answer may be different for different species.

Populations of *Gasteracantha cancriformis*, a brightly coloured and thorny spider, do not seem to be affected by lizards; while *Argiope argentata*, a species readily taken by lizards, particularly lacks juveniles in its populations on lizard islands (juveniles build their webs closer to the ground and are more susceptible to predation). These two species suggest that predation is the cause but for *Eustala cazieri*, another edible species, there is again no effect of presence of lizard density, possibly because this species feeds by night as well as by day, which should reduce competition with lizards. Unravelling the effects of competition and predation is difficult because of the extreme variability in the population density of the spiders, which may also make their populations particularly prone to extinction.

Extinction has greatly affected the species list of birds on Barro Colorado Island, formed by the building of the Panama Canal at the beginning of the century. Between 15 and 60 species may have gone extinct compared with 161 breeding species known today⁵. By extensive use of mist net studies to determine population densities both on the island and on the mainland, Karr⁶ shows very neatly that, in general, the species that might be expected on the island, but that are not there, are those with particularly variable population densities on the mainland. Population variability seems both for these birds and for the Bahamian spiders to be more important than average population size as a predictor of extinction.

Whether extinction or an inability to immigrate determines the lower limit of size for an inhabited island for any species, as shown by incidence curves, again requires knowledge of population dynamics. Schoener and Schoener⁷ have experimented with populations of lizards (again mostly *A. sagrei*) on Bahamian islands. Islands of size down to 167 m² have lizards, with population densities typically between 1–10 m² and 1–100 m². After the introduction of five to ten lizards, on islands varying in size from less than 1 m² to 1 hectare, populations persisted for five years on all islands of 13 m² or more but were extinguished within days on islands of 4 m² or less.

The transition from extinction to persistent populations plotted against island area is remarkably sharp, and occurs at an area of about an order of magnitude less than found for natural populations. Perhaps hurricanes are responsible: populations of small islands may become extinct in storms, or lizards may only immigrate in the wake of storms.

Another way of looking at population persistence has been devised by Haila and Järvinen⁸. Instead of drawing incidence curves showing the presence or absence of particular species on islands of different sizes they have derived prevalence functions showing the population densities. On islands in the Åland archipelago in the Baltic, this technique brings out subtle differences in habitat requirements of two warblers, the Whitethroat (*Sylvia communis*) and the Blackcap (*S. atricapilla*), the former having increased densities on small islands where the latter is almost absent. The cause seems to be the scrubby nature of the forests on the islands, with the trees only 10–12 m high compared with 16–18 m on the mainland.

Prevalence functions also bring out the relative suitability of different habitats for fieldfares (*Turdus pilaris*). When the population crashes, the island habitats are the more affected.

As new theories are developed to replace the popular but obsolete MacArthur and Wilson theory⁹, population dynamics will be a key feature, making island biogeography even more relevant to conservation than it has been¹⁰. □

Mark Williamson is Professor in the Department of Biology at the University of York, York YO1 5DD.

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Gene regulation

Antics of the elusive trypanosome

from Mervyn Turner

THE survival strategy of the African trypanosome has of late provided fascinating insights into the ways in which eukaryotic gene expression can be controlled. The trypanosome survives in the bloodstream of its mammalian host through a series of changes in its surface which is composed of a matrix of identical glycoprotein molecules known as variant surface glycoproteins (VSGs). Expression of different VSG genes in the course of the infection allows the parasite to parade a sequence of antigenically distinct variants past the host's immune system, which is unable to keep pace with it. The mechanism by which VSG gene expression is regulated provided one of the principal areas of discussion at a recent meeting in Utah*.

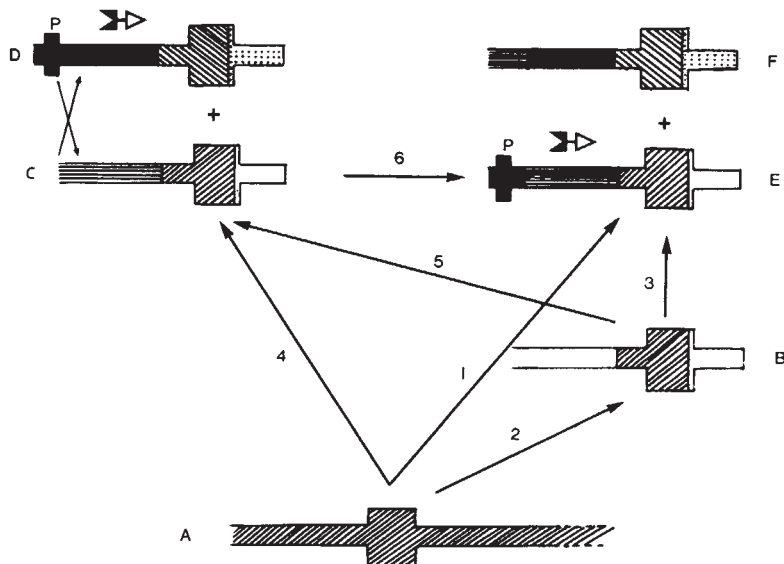
Two apparently different mechanisms have been reported by different groups (reviewed in refs 1–3). Some VSG genes seem to be activated by duplication of the so-called basic copy of the gene (BC) to form an additional expression-linked copy (ELC) which is then transposed to a different site in the trypanosome genome. The ELC is found only in the genome of trypanosomes expressing that particular antigen. Such behaviour suggests that antigenic variation may be the consequence of sequential gene conversion events in which each succeeding ELC is replaced by a new ELC generated from a different BC.

However, not all VSG genes show such behaviour. Some display a stable number of gene copies at all times, with no duplication or detectable gene rearrangements correlated with expression. Any model for the control of antigenic variation must explain both ELC and non-ELC VSG gene behaviour, and much discussion centred on this point.

Expressed VSG genes of both ELC and non-ELC types are always located in a very characteristic region in which the VSG gene is flanked by long barren sequences of 5–15 kilobases (kb) devoid of restriction enzyme sites. On the 3' side of the gene, the barren region terminates at a cluster of restriction enzyme sites which has been identified as the end of a chromosome^{4,5}. Analysis of the structure of this telomeric site has been hampered by the difficulty encountered by all groups in cloning such featureless DNA.

Another characteristic of this 3' telomeric sequence is that its length is highly variable. The variation here, however, seems to be independent of gene switching (P. Borst, University of Amsterdam) and may be an essential feature of chromosome ends in general, possibly related to the mechanisms by which telomeres are replicated (see ref. 6).

The barren sequence at the 5' side of expressed VSG genes is also variable in length



Possible pathways of VSG-gene activation. 1, A non-telomeric VSG gene basic copy (A) is duplicated and transposed into an expression site (E) by gene conversion, producing an ELC. This pathway could also operate through two gene conversion events (2+3), through an intermediate telomeric VSG site, on a minichromosome or other acceptor site (B). Gene conversion could also produce a non-ELC gene (C), either directly (4) or through the intermediate B (2+5). Non-ELC genes are expressed following reciprocal end-of-chromosome exchange with a VSG gene in a unique expression site (D), at a site a long way upstream from the 5' end of the gene (6) to produce an expressed copy (E) and a lingering ELC (F). Thicker regions denote sequences found in VSG mRNA and shading represents the source of the sequences that are found at the expression site. The promoter is indicated by the black bar, P, and the arrow signifies transcription.

in ELCs, but in this case length variation seems to occur only during gene switching (Borst) — possibly through variable alignment of repetitive DNA sequences during the gene conversion that is believed to mediate the switch. Now for the first time, some sequences from a 5' fragment cloned from an ELC have been obtained and compared with sequences derived from the BC. The 5' recombination boundary of the ELC transposed segment has been located within a sequence 700 base pairs (bp) upstream from the VSG-coding sequence, and which is reiterated throughout the genome (J. Donelson, University of Iowa). This sequence presumably corresponds to a 70-bp sequence repeated up to five times on the 5' side of BCs (Borst), and which seems to be associated with all VSG genes⁷.

Mapping studies of ELCs and BCs have shown that up to 2 kb of 5' sequence are co-transposed with the VSG gene. However, the transcriptional start signal cannot lie within the transposed segment, because the sequence lacks 35 bases found at the 5' end of VSG mRNAs⁸. This is true for mRNAs transcribed not only from ELCs, but also from non-ELC VSG genes (J. Boothroyd, Stanford University). These 35 bases must thus be encoded in a 5' exon; but the length of the intron separating it from the rest of the coding sequences remains uncertain: mapping studies failed to locate the 35-bp sequence within 30 kb of the 5' end of a telomeric VSG gene. Genomic Southern blots have shown, remarkably, that the sequence may be present in a single tandem array of up to 200 copies. Borst has suggested that if the 35-bp sequence were associated with a promoter sequence, such an array could function to concentrate polymerase molecules and ensure high levels of transcription of VSG genes from a unique expression site, generating very large transcripts (30 kb). To date, the largest VSG-specific transcript identified is about 8 kb (N. Agabian and M. Parsons, University of Washington, Seattle). Clearly much work on the distribution and role of the 35-bp sequence is needed to test this hypothesis.

A clear corollary to this hypothesis is that since transcripts from both ELCs and non-ELCs have the 35-bp sequence, then both types of gene must fall under the control of the same promoter. Borst pointed out that if this promoter lies some 50–100 kb upstream of the characteristic telomeric expression site, different VSG genes could be brought under the control of the putative unique promoter by the reciprocal exchange of chromosome ends containing VSG genes. Such large-scale transpositions would not be detected by conventional Southern blotting experiments; and they would also explain why the activation of a non-ELC gene does not always result in the disappearance of the ELC gene associated with the immediately preceding variant an-

*Molecular Biology of Host-Parasite Interactions'. UCLA Symposium, Park City, Utah; 30 January–4 February 1983.

tigen. Clearly, this 'lingering ELC' could then be re-expressed without duplication, by a similarly invisible transposition.

Re-expression of a VSG gene associated with a lingering ELC has in fact been seen in *Trypanosoma equiperdum*, but in this case apparently by a second duplicative transposition (H. Eisen, S. Longacre and G. Buck, Institut Pasteur, Paris). Indeed, the new ELC could be mapped into four different telomeric sites in four different trypanosome lines. This could mean that there are multiple expression sites. But a more baroque alternative is that there can be more than one step in the progress of a given VSG gene to a unique expression site, and that the expressed copy in this case was transposed to different intermediate sites in each of the four lines before the final transposition that resulted in expression.

Further evidence for an intermediate site is to be found in some recent work on the ILTAR 1 serodeme of *Trypanosoma brucei* and a related serodeme, ETAR 1. (A serodeme is a population of trypanosomes, each of which can express the same range of variant antigen types.) All the different variant antigen types which make up the ILTAR 1 serodeme of *Trypanosoma brucei* contain five copies of the ILTat 1.3 gene, whether or not this gene is being expressed — that is, it is a typical non-ELC gene. Three copies are in non-telomeric basic-copy environments and two are telomeric. Of the two telomeric copies, one is on a linear DNA molecule of about 100 kb termed the minichromosome; but it is the second telomeric copy which becomes activated when the ILTat 1.3 gene is expressed (as judged by sensitivity to DNase I). Mapping and sequencing studies established that the two telomeric copies must have arisen by duplicative transposition of a sequence extending from beyond the 5' end of the gene to within the 3' end of the gene (as found for ELC genes); but whether one of the telomeric VSGs served as the precursor of the other, and if so which one, could not be determined.

Some clues were however forthcoming from the related serodeme, ETAR 1. Most ETAR 1 clones contain four copies of the ILTat 1.3 gene, but lack the expressible fifth copy. But one clone, ETat 1.8, expresses a VSG whose product is serologically indistinguishable from that of ILTat 1.3; and this clone contains the extra telomeric copy. This has been generated by duplicative transposition from the minichromosome of a sequence extending

from beyond the 5' end of the VSG gene possibly as far as the telomere on the 3' side. Hence, generation of an expressible VSG gene requires two duplicative transpositions (R.O. Williams, ILRAD, Nairobi).

If this is a general mechanism for the production of telomeric VSGs, including ELCs, all the gene patterns observed in Southern blots can be explained by different rates of duplicative transposition into different telomeric acceptor sites. Since 50 or more generations elapse between the gene-switching event and its analysis, an intermediate copy that was frequently replaced would not be seen as an additional telomeric VSG. ELC, lingering ELC, or non-ELC behaviour would thus reflect the rate of replacement of VSG genes in the site allowing expression.

In some cases, non-telomeric BCs seem

to be replaced by telomeric ones. In these cases, perhaps the non-telomeric copy is lost after having been duplicated to produce a telomeric 'basic copy' (that is, stable intermediate) which by duplicative transposition could produce an ELC, as recently observed⁹. Whether such an intermediate always exists, and if so whether it is always on a minichromosome, remains to be established. Other VSG genes on minichromosomes have, however, been reported by Borst.

The possible pathways by which VSG genes may be activated are summarized in the figure. There may be more still. □

Mervyn Turner is at the Molteno Institute, University of Cambridge, Downing Street, Cambridge CB2 3EE.

Morphogenesis

Two signals to shape a slime mould

from James H. Morrissey

THE search for morphogens — the chemical signals responsible for organizing and regulating form and pattern in biology — has been plagued by false leads. This is partly because of the complexity of most embryos, but it is also because of the bizarre array of simple and unrelated chemicals that can act as very efficient embryonic inducers¹. Notable exceptions have been the identification of endogenous morphogenetically active substances from *Hydra*^{2,3} and the remarkable finding that vitamin A can cause a complete and well organized respecification of positional values in vertebrate limb buds⁴. The cellular slime mould *Dictyostelium discoideum* has always held great promise as a nearly ideal model system in which to study morphogens, and its potential may now have been realized in two exciting reports in this issue of *Nature*^{5,6}.

The proportions of the two major cell types in *Dictyostelium* fruiting bodies, spores and stalk cells, are established soon after the amoebae have aggregated together and result in a simple pattern of precursor cells in the migrating slug, with prestalk cells in the front fifth and prespore cells behind. Cyclic AMP, the chemo-attractant for aggregation, has been proposed to induce the stalk pathway of differentiation, but now it seems that, although required for later differentiation, cyclic AMP is in fact pathway-indifferent⁷.

An important advance in the study of slime mould morphogens came with the use of mutant strains of slime mould that can be induced to differentiate in the presence of cyclic AMP without aggregating into a slug⁸. This enabled the Mill Hill group to show that the density dependence of stalk differentiation could be overcome by ad-

ding back a crude exudate released from developing cells, and they named the active substance differentiation-inducing factor (DIF). Spore formation, however, was found to be density-independent⁹.

In the first of this week's papers, Kay and Jermyn⁵ show that highly purified DIF not only supports stalk differentiation, but also can induce redifferentiation of prespore cells into stalk cells. Although DIF is active at remarkably low concentrations *in vitro* (less than 10⁻¹⁰ M) and is first detectable at the developmental stage when cell-type-specific differentiation is thought to begin¹⁰, a nagging question has been whether it has the same action *in vivo*. Strong evidence in favour of such a role for DIF now comes from a study of mutants lacking extractable DIF activity¹¹. They develop prespore cells but not prestalk cells and thus have a grossly abnormal later morphogenesis. Significantly, adding exogenous DIF corrects the mutant phenotype by allowing stalk cell differentiation.

Dictyostelium cells secrete ammonia in millimolar quantities, which serves to control the choice between continued slug migration and the construction of the fruiting body. Ammonia has also been postulated to control the choice between spore and stalk differentiation by antagonizing cyclic AMP production¹², but this seems unlikely because it entails the assumption of a pathway-specific role for cyclic AMP.

The Mill Hill group has resurrected ammonia as a second morphogen along with DIF in the second of this week's papers. They propose that the two act antagonistically to affect intracellular pH, which in turn controls the choice between

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alternative pathways of differentiation⁶. Ammonia can do this because it is a permeant weak base, but DIF, they suggest, acts by blocking a cell-surface proton pump. This is supported by, among other things, the exciting new finding that low concentrations of diethylstilbestrol, a potent inhibitor of proton pumps in fungi, can efficiently induce stalk formation. It remains to be demonstrated however that such pumps exist in *Dictyostelium* and are modulated by DIF levels; furthermore it will be necessary to determine the structure of DIF, and to find out how (and indeed whether) DIF and ammonia concentrations can carry positional information. But to be able to formulate such specific questions at all is a unique achievement in the field of pattern formation.

Recent evidence from several groups complicates any straight-forward morphogenetic gradient explanation of slime mould pattern formation. Two years ago, Sternfeld and David¹³ discovered a third minority cell type in *D. discoideum* slugs which shares staining and ultrastructural properties with prestalk cells. These cells, the 'anterior-like' cells, are dispersed throughout the posterior (prespore) zone. Anterior-like cells sort out to form a new tip when the prestalk zone is removed; this is presumably prevented in normal slugs by a diffusible inhibitor released by prestalk cells. Some of the remaining prespore cells quickly redifferentiate to replenish the supply of anterior-like cells, and in fact the new prestalk zone is made up partly of redifferentiated prespore cells¹⁴.

Since pattern regulation, in prespore isolates at least, seems to involve sorting out of randomly dispersed cell types, it seems possible, by analogy, that the original pattern is formed by random differentiation followed by sorting out. This idea, first promulgated by Takeuchi¹⁵, has received some support from the finding that prespore cells are first found to be intermingled with non-prespore cells during the late aggregate stage^{16,17}. Unfortunately, it is not yet possible to distinguish between prestalk cells and undifferentiated

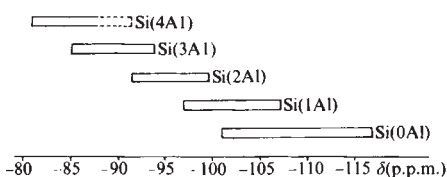
cells in such aggregates, and it would be necessary to demonstrate actual intermingling of both cell types as they differentiate to rule out positionally specified differentiation. Prestalk cells do make specific proteins¹⁸, so it is hoped that monoclonal antibodies will soon provide the tools necessary to settle this question. But even in the case of such salt and pepper patterns, diffusible substances would still be a plausible basis for the control of cell proportions: only the effective range of the morphogens would be shorter.

Zeolites

Bond angles and chemical shifts

from Lovat Rees

ZEOLITES are porous tectosilicates of immense importance as catalysts and molecular sieves in the petroleum industry. Recently much interest has been focused on the short-range Si-Al order in the zeolite framework, since this appears to be fundamental to the catalytic and other chemical properties. Thermal stability, important for industrial applications, is known generally to increase with increasing Si/Al ratio, and to this end aluminium can be removed from the framework either by treatment with SiCl_4 or by heat treatment of the ammonium-exchanged zeolite, without disrupting the original topology and crystallinity of the parent material.



Overlapping shift ranges of different aluminosilicate networks, $\text{Si}(n\text{Al})$, where $n = 0-4$.

The latter process is widely used to produce 'ultrastable' faujasite — the catalyst used by all major oil companies for cracking and hydrocracking reactions in petroleum refining.

Since the first study of zeolites by high-resolution solid-state ^{29}Si nuclear magnetic resonance (NMR), described in 1979 (ref.1), considerable progress has been made in elucidating the structure of both natural and synthetic zeolites using the technique. In the study of Si-Al structure the method gives distinct chemical shifts for SiO_4 tetrahedra, depending on how many AlO_4 tetrahedra they are linked to via oxygen bridges. It is also possible to estimate the relative numbers of the various $\text{Si}(n\text{Al})$ (where $n = 0-4$) links from signal intensities.

However recent studies of some thirty natural and synthetic zeolites² have shown that as well as the number of neighbouring aluminium atoms, other structural features, such as bond angle, cation type

and position, may induce fairly large shift contributions. Therefore, the shift ranges of $\text{Si}(n\text{Al})$ units with different values of n may overlap significantly for different types of aluminosilicate network (see the figure) and the assignment of n from chemical shifts alone may lead to erroneous conclusions^{3,4}.

James H. Morrissey is at the Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU.

In this issue of *Nature*, Smith and Blackwell (p.223) have tested empirical correlations between the chemical shift for aluminium-free silicates (that is, $\text{Si}(n\text{Al})$ with $n = 0$) and several geometrical parameters in a number of silica polymorphs. They find strong correlations between chemical shift and mean Si-Si bond length and with mean Si-O-Si bond angle, and a weak correlation with mean Si-O bond distance. These results suggest that we are now closer to a more exact understanding of the reasons for the spread of chemical shifts, and confirm an earlier suggestion by Thomas *et al.*⁵ that a qualitative correlation should exist between chemical shifts and T-O-T bond angles (where T = Si or Al) in zeolite frameworks.

With a knowledge of atomic coordinates of a zeolite structure, unambiguous assignments of n may become possible even for those zeolites that show only a single NMR resonance. For example, the recently published ^{29}Si NMR spectrum of calcined silicalite containing nine different chemical shifts⁶ has been confirmed by Smith and Blackwell. But because the atomic coordinates were not known with sufficient accuracy Smith and Blackwell could not assign the lines to specific SiO_4 tetrahedra.

Lovat Rees is in the Department of Chemistry, Imperial College of Science and Technology, London SW7 2AZ.

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Isotope geochemistry of Cenozoic volcanic rocks reveals mantle heterogeneity below western USA

Martin A. Menzies*, William P. Leeman† & Chris J. Hawkesworth*

* Department of Earth Sciences, Open University, Milton Keynes MK7 6AA, UK

† Department of Geology, Rice University, PO Box 1892, Houston, Texas 77001, USA

Isotope geochemistry of Cenozoic volcanic rocks from the western USA demonstrates considerable mantle heterogeneity in the source regions. The Basin and Range volcanic rocks have tapped a source similar to mid-oceanic ridge basalts and the Hawaiian islands, while the magmas erupted in the Sierran Province formed in enriched grossly heterogeneous garnet bearing mantle. Within the bimodal rhyolite–basalt province of the Snake River Plain and Yellowstone National Park, there exist olivine tholeiites that have retained the primary isotopic characteristics of their mantle source area.

THE isotopic and chemical composition of the sub-continental lithosphere remains the subject of considerable debate and speculation. Continental cratons are coupled to underlying mantle that moves with it with respect to convecting upper mantle¹. The resultant isolation of this sub-continental mantle from the effects of large scale convection preserves a unique record of magmatic and metasomatic events spanning much of Earth's history. Efficient and perhaps more frequent convective overturn in the sub-oceanic lithosphere may obliterate much of this record. Random samples of the sub-continental (and sub-oceanic) mantle are rapidly transported to the surface in volcanic diatremes and kimberlite pipes. Both the host volcanic rock and the ultramafic fragments carry critical information about chemical and isotopic inhomogeneities in their source regions. One of the principal conclusions from studies of spinel and garnet peridotites is that gross heterogeneities have developed below continents in response to local perturbations in the Rb/Sr and Sm/Nd systems^{2–6}. However, the sub-oceanic regions tend to be more homogeneous⁷. Although some would argue that the genetic relationship between ultramafic rocks and recent basaltic volcanism is at best equivocal, their chemical and isotopic variability must somehow be reconciled with magmatogenesis. Volcanic rocks, therefore, provide important complementary evidence on the nature of the sub-continental mantle. However, because they may interact with continental crust on their way to the surface, the effects of such crustal contamination must be assessed and eliminated before the compositions of their source regions can be discussed.

On a plot of $^{143}\text{Nd}/^{144}\text{Nd}$ against $^{87}\text{Sr}/^{86}\text{Sr}$ ($\epsilon_{\text{Nd}} - \epsilon_{\text{Sr}}$) some continental volcanic rocks plot within the 'mantle array' as defined by uncontaminated oceanic basalts, but many plot outside this compositional field⁷. Samples plotting within the 'array' at high ϵ_{Nd} and low ϵ_{Sr} are generally accepted as being mantle derived since their source regions are similar to mid-oceanic ridge basalts (MORB) and oceanic islands. Note, however, that some continental flood basalts that are chemically and isotopically indistinguishable from oceanic alkaline magmas are believed by some to have interacted with crust⁸. Flood basalts tend to have a wide range of ϵ_{Nd} and ϵ_{Sr} and this has led to conflicting viewpoints about their origin. De Paolo and Wasserburg⁸ invoke magmatogenesis from within an unfractionated chondritic mantle source region whereas Carlson *et al.*⁸ prefer modification of MORB type magmas by combined assimilation and crystallization. Any hypothesis appealing to contamination as a viable mechanism in the genesis of flood tholeiites must explain the vast volumes of homogeneous lava extruded from various volcanic fissures. Heterogeneous mantle that has evolved in response to variable Rb/Sr and Sm/Nd over billions of years would seem to be a more suitable source for uniform tholeiitic and alkaline magmatism than chemically and isotopi-

cally heterogeneous crust. In this context Carlson *et al.*⁸ agree that a source for flood basalts in heterogeneous mantle cannot be 'unambiguously excluded' although their preference is a model involving crustal contamination. Moreover, Hawkesworth *et al.*¹⁰ have linked the high initial $^{87}\text{Sr}/^{86}\text{Sr}$ and low $^{143}\text{Nd}/^{144}\text{Nd}$ ratios which are typical of most of the Karoo basalts of southern Africa with the stabilization of trace element enriched upper mantle shortly after locally important orogenic events at 1,400–1,000 Myr. Interpretations are perhaps most divergent when dealing with volcanic suites with radiogenic Sr and non-radiogenic Nd which plot to the right of the 'array'. Many authors have invoked bulk crustal assimilation to explain such relatively high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios⁸, but they can develop in comparatively short periods of time in high Rb/Sr mantle xenoliths⁴. Consequently the issue is not whether crustal contamination and isotopically and chemically enriched mantle do occur, for there is plenty of evidence in support of both^{3–5,7–12}, but how they should both be recognized. We now report Sr and Nd isotopic analyses from a volcanic field within the Basin and Range province (Arizona); the Sierran Province (California and Nevada) and the Snake River Plain–Yellowstone National

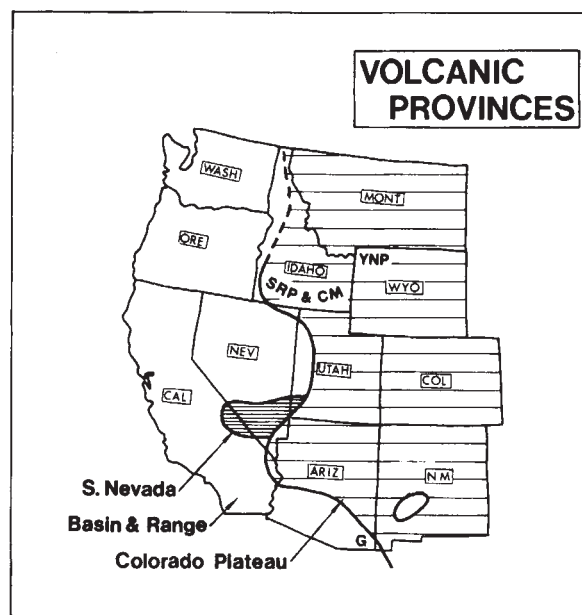


Fig. 1 Cenozoic volcanic provinces in the western USA^{20,22}. Areas of interest are: G, Geronimo volcanic field (S Basin and Range); Sierran Province; SRP, Snake River Plain; CM, Craters of the Moon; YNP, Yellowstone National Park.

Table 1 Cenozoic volcanic rocks from the western USA

South Basin and Range Province	Rb/Sr*	⁸⁷ Sr/ ⁸⁶ Sr	¹⁴³ Nd/ ¹⁴⁴ Nd
Geronimo Volcanic Field			
76-6	Alkali olivine basalts	0.70296	0.513021 ± 8
Pdk-34		0.70304	0.513033 ± 20
RF-26		0.70295	0.513031 ± 18
90-4		0.70321	—
76-9		0.70289	—
Pdk-56		0.70285	0.513037 ± 10
90-8		0.70327	0.513025 ± 20
Pisgah Crater and Baja California			
PCB-1 (ref. 19)		0.70346	0.51294
CSQ-3 (ref. 38)		0.70301	0.51297
Sierran Province			
67-22		0.70686	0.512145 ± 16
67-25		0.70689	0.512147 ± 34
67-33		0.70597	0.512468 ± 18
67-43		0.70473	0.512725 ± 18
67-45		0.70572	0.512617 ± 10
67-60		0.70582	0.512375 ± 10
67-61		0.70601	0.512524 ± 12
67-69		0.70485	0.512676 ± 10
Snake River Plain–Yellowstone National Park			
70-3	0.057	0.70656	0.512483 ± 32
74-26	0.025	0.70655	0.512499 ± 14
SBR-3	0.046	0.70630	0.512442 ± 20
74-CB	0.010	0.70753	0.512455 ± 14
69-G	0.034	0.70585	0.512522 ± 22
70-16	0.028	0.70788	0.512366 ± 10
69-56	0.060	0.70666	0.512401 ± 8
69-49	0.057	0.70671	0.512431 ± 18
67-85	0.060	0.70729	0.512368 ± 20
SBR-6	0.045	0.70672	0.512395 ± 10
69-47	0.026	0.70661	0.512443 ± 54
69-10	0.011	0.70714	0.512433 ± 8
6YC-142	0.027	0.70601	0.512460 ± 14
6YC-136	0.023	0.70578	0.512483 ± 6
P104	0.045	0.70657	0.512412 ± 38

* Rb and Sr determinations from Leeman (unpublished data).

Park bimodal basalt–rhyolite province^{13–13} (Idaho and Wyoming) (Fig. 1).

Sr-isotope ratios were determined using standard ion exchange techniques on a VG-Micromass 54E thermal ionization mass spectrometer controlled by microcomputer. All $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are measured to 0.005% or better (2 s.e.m.) and NBS 987 = 0.71015 ± 2 . Nd was separated using a cation and then a temperature controlled anion column procedure and then run as the metal species on a triple filament assembly. $^{143}\text{Nd}/^{144}\text{Nd}$ is normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$ and three analyses of BCR-1 = 0.512632 ± 8 , 0.512617 ± 30 and 0.512634 ± 8 . Representative Sr and Nd isotopic analyses are given in Table 1, and all the data are plotted on Figs 2 and 3.

South Basin and Range Province

The Geronimo volcanic field of the San Bernardino Valley (southeastern Arizona) comprises lava flows, maars and cinder cones of mildly silica-undersaturated alkali olivine basalt with Mg/Mg + Fe ratios in the range 0.65–0.55. Volcanism is related to the change in plate motions from compressional to tensional, and may have been triggered by diapirism above a subducted plate^{17,18} (Fig. 1).

Alkali olivine basalts (AOB) are characterized by a uniform major element chemistry and a light rare-earth element (LREE) enriched profile. Representative samples of the AOB have radiogenic Nd and non-radiogenic Sr isotope compositions (Figs 2 and 3, Table 1) and are in many respects similar to E-type MORB and alkalic-nephelinitic lavas from Hawaii⁷ (Fig. 2). Our results for the Geronimo lavas compare favourably with available Sr–Nd analyses from elsewhere in the Basin and Range Province (Pisgah Crater, California¹⁹) (Table 1).

However, the range in $^{87}\text{Sr}/^{86}\text{Sr}$ reported by Hedge and Noble²⁰ tends to be slightly more radiogenic. Taken together these data indicate derivation of LREE enriched alkaline magmas at Geronimo and elsewhere in the South Basin and Range from a mantle source region with a depletion in Rb/Sr and LREE (high Sm/Nd). Extraction of a light-ion lithophile (LIL) element enriched fluid from mantle depleted for possibly billions of years requires either unacceptably low degrees of melting or a recent change in the Rb/Sr and Sm/Nd regime not yet registered in the isotopic ratio. For further evidence of potentially important mantle events beneath the US we can turn to chemical and isotopic investigations of mantle xenoliths from this area.

Type I and II mantle nodules¹⁸ and megacrysts of pyroxene, feldspar and amphibole are present in most flows and cinder cones at Geronimo and at various other localities throughout the southwestern USA. Minerals separated from these nodules exhibit a considerable range in ϵ_{Nd} (+13 to –1.0) which is much larger than would be expected if they were simple residua related to genesis of the host alkaline magmas¹⁸ (Fig. 2). Clearly the ultramafic rocks found within the AOB are random time capsules of a variety of mantle events spanning a greater mantle residence time than that of recent magmatism. Local impregnations of trace element enriched Fe-rich fluids are evident as an amphibole $\pm$ apatite $\pm$ mica stockwork in many of these nodules. Isotopic analyses of the mineral phases in these veins reveals a depleted character that is inconsistent with their LREE enriched character. This can be taken as evidence that the 'veining event' occurred recently, but it remains to be seen whether or not the veining represents the vital precursory modification of the alkali basalt source invoked in the preceding section, or merely apophyses surrounding a deep-seated magma conduit¹⁸. Volcanic rocks from the Basin and Range have lead ratios²² ($^{207}\text{Pb}/^{204}\text{Pb}$; $^{206}\text{Pb}/^{204}\text{Pb}$; $^{208}\text{Pb}/^{204}\text{Pb}$) comparable to volcanic rocks from oceanic and volcanic arc settings. This feature in conjunction with the extremely narrow range in Sr and Nd isotopic composition, points to more of the Basin and Range lavas as being ultimately mantle derived magmas. Their source regions appear to have been similar to those of E-type MORB or oceanic island basalts and there is no evidence of any significant contamination with crustal material. The ubiquitous association of ultramafic xenoliths of varied sizes in many of the alkalic basalts (high Mg values) supports this assertion, because such lavas are likely to have ascended through the crust fairly rapidly allowing minimal interaction with crustal wall-rock.

Sierra Nevada Province

Within the Basin and Range Province (Fig. 1) there occurs a region of relatively thick crust (≈ 50 km) that coincides with the northern edge of the Precambrian craton. Volcanism within this province, the Sierran or Sierra Nevada Province (SNP), includes alkali olivine basalts, trachybasalts, and potassic lavas^{21,22}. The bulk of this alkaline magmatism occurred in a region where subduction had ceased a few million years earlier. Cessation of a subduction related regime was followed by initiation of extensional tectonics characterized by normal faulting. Volcanism developed in response to this new tectonic framework.

Alkali olivine basalts from California and Nevada have a wide range in $^{87}\text{Sr}/^{86}\text{Sr}$ (0.70473–0.70689) and $^{143}\text{Nd}/^{144}\text{Nd}$ (0.512725–0.512145) (Fig. 2). These lavas plot on the 'mantle array' as defined by oceanic lavas, and extend it to higher $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (Fig. 3, Table 1). Previous workers have reported a similar range in Sr isotopic composition but more importantly they have commented on the unusually high Sr contents (700–2,500 p.p.m.) of Sierran Province lavas²⁰. The high relative abundance of Sr and the lack of any negative correlation between Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ argue against significant crustal involvement during magmatogenesis²⁰. Hedge and Noble²⁰ estimated that it would require >40% of crustal lithologies to

modify the Sr isotopic composition of the SNP lavas; this is clearly inconsistent with the recorded Mg values and incompatible element contents. To illustrate this further mixing hyperbolae were calculated between end member liquid compositions close to the bulk Earth and crustal compositions similar to gneissic and granulitic xenoliths from elsewhere in the western USA (M.A.M. and W.P.L., unpublished work). Although the volcanic rocks conform to the hyperbolae the hypothetical liquids have significantly lower Sr (<200 p.p.m.) than the SNP magmas. Invoking a more Sr rich crustal endmember does not improve the situation as hyperbola become more convex and so fail to fit the observed isotopic data. According to Van Kooten²¹, a significant amount of crustal mica (45–85%) could have been assimilated or somehow mobilized by 'selective contamination' processes to produce the high K₂O contents of the volcanic rocks. Removal of a wall-rock melt fraction and incorporation of this into a mantle melt would presumably be apparent on an Rb/Sr versus ⁸⁷Sr/⁸⁶Sr plot, and yet Hedge and Noble²⁰ have already commented on the lack of such a positive correlation. Similarly the lack of any 'apparent Pb isochrons' in the volcanic rocks²², and the limited local variation in Pb isotopic ratio would argue against involvement of Precambrian heterogeneous cratonic rocks. Furthermore Eversen²² noted that rhyolite and basalt lead data cannot be reconciled with a crustal contamination model. Similarly Van Kooten²³, in a study of Pb and Sr isotopic geochemistry of basaltic and ultrapotassic rocks from the Sierran Province, stressed the lack of any well defined mixing trends as would be expected if contamination had occurred. Moreover, certain of the conditions necessary for binary mixing are not satisfied by the data²³. Significant scatter is evident in 1/Pb versus Pb isotopic composition²² and 1/Sr versus ⁸⁷Sr/⁸⁶Sr, and any systematic trends which might be expected from crustal involvement are lacking.

These isotopic and trace element considerations suggest that heterogeneity of the mantle source region is a prerequisite in the magmatic evolution of the Sierra Nevada Province. Such inhomogeneities in hydrous garnet peridotite could adequately explain the positive correlation observed between Sr and ⁸⁷Sr/⁸⁶Sr. Rb/Sr and Sm/Nd ratios of migrating fluids integrated over time produce extreme Sr and Nd isotopic variability in the confines of the sub-continental mantle (>1,000 Myr)^{5,6}. Nd and Sr isotopic studies of garnet peridotites confirm this hypothesis with a record of extreme variability in isotopic composition locked in mantle mineral phases⁵ (Figs 2 and 3). Moreover, the chemistry of phlogopite bearing mantle can, on application²¹ of partial melting calculations, accurately reproduce the chemistry of Sierran lavas. This led Van Kooten²¹ to postulate a mica-garnet clinopyroxene rich source region below the Sierra Nevada, and to invoke extensive enrichment of the upper mantle. Essentially he outlined a two-stage process whereby a mantle peridotite is gradually transformed to a clinopyroxene rich assemblage by major element influx. Migration of partial melt fractions through a type I peridotite leads to its conversion to a clinopyroxenite as a result of metasomatic processes. The second stage of Van Kooten's model involved an overall alkali earth enrichment reflecting perhaps pervasive metasomatic activity responsible for development of a mica-amphibole stockwork in type I peridotites. Mantle degassing and LIL element mobility may be coincident with Sierra Nevada uplift and an overall change in tectonic regime. We reiterate these conclusions and propose an origin for the Sierra Nevada Province volcanic rocks in enriched grossly heterogeneous (hydrous?) garnet peridotite. Everson²² proposed that Pb isotopic variations in this province reflect gross mantle heterogeneities that have evolved over billions of years (>1,500 Myr) in the relative isolation of sub-cratonic regions. Furthermore, Hedge and Noble²⁰ tentatively suggested a 1,500 Myr event in the source regions of these volcanic rocks based on Rb–Sr data. These age constraints are compatible with the timing of enrichment events calculated for mantle source regions assuming a simple two-stage model²⁴ for Nd and Sr isotopes.

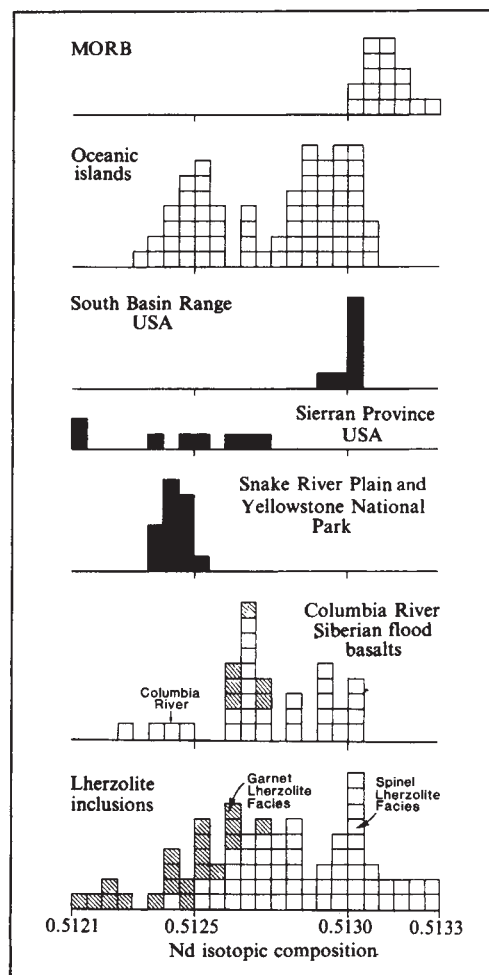


Fig. 2 Nd isotopic variation in continental and oceanic volcanic rocks relative to data for lherzolite xenoliths. Note that both oceanic and continental basalts record a source heterogeneity compatible with that observed in mantle ultramafic rocks. Taken as a group, flood basalts tap extremely inhomogeneous source regions and do not have unique source areas with either ¹⁴³Nd/¹⁴⁴Nd ≈ 0.51262 or ¹⁴³Nd/¹⁴⁴Nd ≈ 0.51300 as has been suggested^{8,9}. Comparative data are taken from various sources: MORB^{31,32}; Oceanic islands^{33–35}; Columbia River^{8,9}; Siberian Flood basalts⁹; and lherzolite inclusions^{5,6,18,29–30,36}.

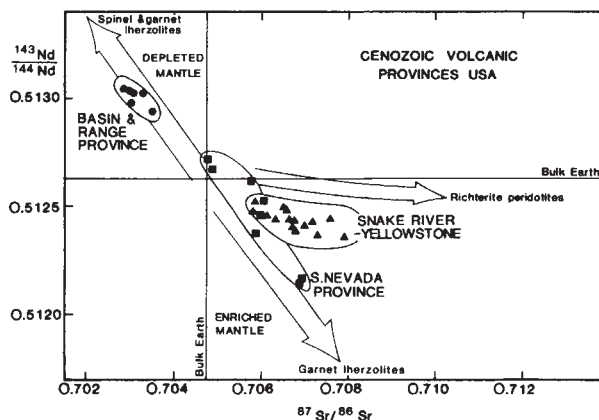


Fig. 3 ¹⁴³Nd/¹⁴⁴Nd versus ⁸⁷Sr/⁸⁶Sr for Cenozoic volcanic rocks from the western USA. Schematic trends for spinel, garnet and richterite bearing lherzolites are taken from various sources^{5,6,18}.

Snake River Plain and Yellowstone National Park

The Snake River Plain (SRP) is an arcuate depression that links the young caldera complexes of Yellowstone and Island Park with the voluminous flood basalts of the Columbia River Plateau to the west^{15,16,25} (Fig. 1). Within its western region the SRP is believed to be a graben bordered by high angle faults whereas in the east it is a downwarp controlled by marginal faults. As well as a structural change, seismic refraction studies reveal mafic crust at 10 km beneath the western SRP and sialic crust in the east adjacent to Yellowstone National Park (YNP). Volcanism within this classic bimodal basalt-rhyolite province began with voluminous eruptions of high silica rhyolite and ash-flows followed by basaltic eruptions. Volcanic activity became progressively younger towards the east and it has been suggested that volcanism is related to migration of the North American Plate over a stationary mantle hotspot presently located beneath the Yellowstone volcanic complex.

The olivine tholeiites have a relatively uniform composition regardless of age or location within the SRP. Generally the basaltic rocks are high TiO_2 , P_2O_5 and Fe lavas with low abundances of silica and alkali earth elements, and are not too dissimilar to E-type MORB¹⁵ (Fig. 4). Variations within this suite of tholeiites can be best attributed to formation of magmas by melting of a mantle source region with subsequent fractionation of olivine and plagioclase at low pressures (<10 kbar). Oxygen isotope data²⁶ are consistent with a mantle origin in that the $\delta^{18}\text{O}$ values converge on a value identical to fresh MORB or ocean island volcanic rocks. The similarity between SRP-YNP tholeiites and some oceanic island basalts is also apparent in the Nd isotopic data (Fig. 2) but it does not extend to the Sr isotopic data. The range in $^{87}\text{Sr}/^{86}\text{Sr} = 0.70788\text{--}0.70578$ in conjunction with $^{143}\text{Nd}/^{144}\text{Nd} = 0.512366\text{--}0.512522$ (Table 1) produces a trend to the right of the 'mantle array' when plotted on an ϵ_{Nd} versus ϵ_{Sr} diagram (Fig. 3). Hence the lavas are either derived from heterogeneous enriched mantle or contaminated with crustal material (for example, amphibolites). Investigation of a contaminated origin of the SRP-YNP tholeiites involved analysis of a wide variety of crustal xenoliths and basement rocks with variable Sr and Nd contents (W.P.L. and M.A.M., unpublished results). This complemented the available major, trace and rare earth element data. We assumed in the mixing calculations that the SRP-YNP tholeiites are a contaminated suite of rocks and must therefore lie on a mixing hyperbola between a mantle derived liquid (such as, MORB) and a crustal endmember. Mixing hyperbolae were computed for mixtures of N and E-type MORB, the latter giving a hyperbola that vaguely approached the trend shown by the SRP-YNP data. Although a 'best-fit' for the isotopic ratios can be produced by extensive modification of the natural crustal compositions, it is impossible to evaluate whether such hypothetical creations are geologically reasonable. More significantly, it is difficult to envisage a crustal process, considering the heterogeneity displayed by xenoliths and basement, that could produce the considerable volumes of homogeneous olivine tholeiites. After consideration of the isotopic data, we believe that the olivine tholeiites have not interacted significantly with the crust and that their Sr-Nd isotopic signature reflects that of the mantle source region. Proponents of crustal contamination^{11,12} need to explain the large and systematic heterogeneities seen in Pb data, while maintaining a narrow range in Sr, Nd and O isotopic composition. The range in Pb, Nd, Sr and O isotopic data^{26,27} that exists in the crust of the varied chemistry (mafic to sialic) known to occur below the SRP negates any simple contamination model. When considered in the context of volcanism tapping mantle source regions residing below stable continents for billions of years, a case can be made for the production of vast amounts of homogeneous tholeiitic magma with $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios respectively lower and higher than those of the bulk Earth⁶. The nature of their source regions is then reflected in

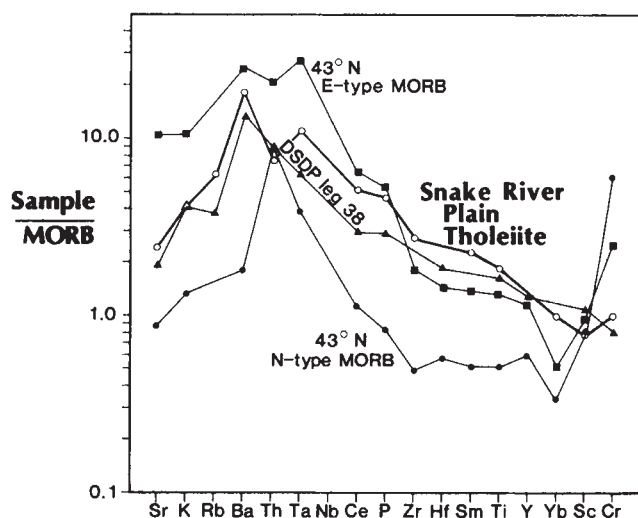


Fig. 4 Elemental variations of Snake River Plain tholeiites (average of 200 analyses^{15,16}) normalized to MORB³⁷. Note the similarity between E-type MORB and these continental tholeiites. In particular note the abundances of K, Rb, Ba, (Th); elements that tend to be dramatically affected by crustal contamination. Interaction of these tholeiitic magmas with continental crust appears to be minimal, a conclusion supported by several other aspects of their geochemistry (see text).

the observed Nd, Sr and Pb isotope composition and the concurrent variations in trace elements (Rb/Sr and Sm/Nd) pertinent to Sr and Nd isotopes. The SRP tholeiites have Rb/Sr ratios consistently lower than associated lavas known to be contaminated^{26,28}. Furthermore most tholeiites have a Rb/Sr > bulk Earth and Sm/Nd < bulk Earth consistent with their high $^{87}\text{Sr}/^{86}\text{Sr}$ and low $^{143}\text{Nd}/^{144}\text{Nd}$ ratios. Sr and Nd isotopic data can be used in conjunction with these trace elements to calculate model Rb-Sr and Sm-Nd ages. In both cases a range of ages are forthcoming and one can infer that these systems record events at 1,000–2,000 Myr. We therefore reiterate the conclusion of Leeman^{15,16} who suggested that Pb (and Sr) data reflect magma genesis through partial melting of ancient ($\approx 2,500$ Myr) enriched sub-continental mantle. The absence of any correlation between $^{87}\text{Sr}/^{86}\text{Sr}$ and major, trace and rare earth element abundances, and the lack of any correlation with $\delta^{18}\text{O}$, $^{206}\text{Pb}/^{204}\text{Pb}$, and $^{208}\text{Pb}/^{204}\text{Pb}$ supports a mantle origin with minimal crustal interaction^{15,16}. Suitable mantle analogues can be found in potassic-richertite bearing peridotites from South African kimberlite pipes⁶ (Fig. 3). Their overall ϵ_{Nd} versus ϵ_{Sr} variation is similar to that of the SRP and YNP tholeiites in that they too have evolved in a low Sm/Nd high Rb/Sr mantle environment.

Conclusion

Cenozoic volcanic rocks from three provinces in the western USA exhibit a considerable range in Sr and Nd isotopes primarily believed to reflect extreme heterogeneity in the sub-continental mantle (Fig. 3).

(1) Basin and Range alkaline magmas ($^{87}\text{Sr}/^{86}\text{Sr} = 0.70285\text{--}0.7033$ $^{143}\text{Nd}/^{144}\text{Nd} = 0.51294\text{--}0.51304$) are derived from a mantle source region identical to that of E-type MORB or the Hawaiian islands. Although depleted in LREE for billions of years, recent LIL element enrichment (≈ 200 Myr) of the source is required to generate alkaline magmas at moderate degrees of melting. Hydrous and anhydrous lherzolites with an isotopic signature identical to the Basin and Range magmas are known from several volcanic edifices^{18,29,30}.

(2) Sierra Nevada Province alkali olivine basalts ($^{87}\text{Sr}/^{86}\text{Sr} = 0.7047\text{--}0.7069$ $^{143}\text{Nd}/^{144}\text{Nd} = 0.512725\text{--}0.51215$) are derived from enriched garnet peridotite. The isotopic variability of the volcanic rocks implies the sub-continental mantle has remained isolated for $\approx 1,500$ Myr. Since then the mantle has been characterized by relatively low Sm/Nd and high Rb/Sr. Garnet lher-

zolites⁵ from kimberlite pipes display isotopic heterogeneity similar to the SNP magmas.

(3) Snake River Plain–Yellowstone National Park olivine tholeiites ($^{87}\text{Sr}/^{86}\text{Sr} = 0.7058\text{--}0.7079$, $^{143}\text{Nd}/^{144}\text{Nd} = 0.51252\text{--}0.51237$) are believed to have been generated by partial melting of ancient ($\approx 2,500$ Myr) sub-continental lithosphere. The isotopic variations evident in the SRP–YNP tholeiites reflect a mantle source that has responded to enrichment events with low Sm/Nd and high Rb/Sr ratios. Amphibole (richterite) lher-

zolites⁶ from South Africa have also evolved in response to high Rb/Sr ratios, ultimately producing a considerable spread in $^{87}\text{Sr}/^{86}\text{Sr}$ ratio.

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Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector

Luis Herrera-Estrella*, Ann Depicker*, Marc Van Montagu* & Jeff Schell*†

* Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

† Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG

Foreign genes introduced into plant cells with Ti-plasmid vectors are not expressed. We have constructed an expression vector derived from the promoter sequence of nopaline synthase, and have inserted the coding sequences of the octopine synthase gene and a chloramphenicol acetyltransferase gene into this vector. These chimaeric genes are functionally expressed in plant cells after their transfer via a Ti-plasmid of Agrobacterium tumefaciens.

CROWN gall formation on dicotyledonous plants by *Agrobacterium tumefaciens* is the result of the transfer and covalent integration of a segment (called T-region) of the Ti-plasmid into the chromosomal DNA of plant cells (for reviews see refs 1–4). Insertion of foreign DNA sequences within the T-region of Ti-plasmids leads to their co-transfer and integration into the plant genome⁵. To date, inserts of up to 50 kilobases (kb) have been successfully co-transferred with the T-DNA (unpublished results of this laboratory). The upper limit of the size of the DNA segments that can be transferred to plant genomes with this system has not been determined. The transferred T-region (or T-DNA) is expressed in plant cells⁶ and codes for several host polymerase II-dependent polyadenylated transcripts^{7–10}. Some of these transcripts were shown to be responsible for the tumorous mode of growth of the transformed plant cells, while others code for enzymes that synthesize novel compounds (termed opines), which are amino acid or sugar derivatives. None of these transcripts was found to be essential for T-DNA transfer^{11–13}.

To understand the mechanism by which T-DNA-linked genes are expressed in plants, the nucleotide sequence and the precise location of 3' and 5' ends of the transcripts of two different opine synthase genes were determined, the octopine synthase gene carried by pTiB6S3 (ref. 14) and the nopaline synthase

from pTiT37 (refs 15, 16). Although both genes are encoded by plasmids of bacterial origin, they share more characteristics with eukaryotic genes than with prokaryotic genes. Both octopine and nopaline synthase genes, designated *ocs* and *nos* respectively, have a sequence similar to the so-called 'TATA' or 'Goldberg–Hogness' box¹⁷ in the 5' region upstream of the start of transcription, and a sequence 'AATAA', similar to the polyadenylation signal of eukaryotic genes, near the 3' end^{18,19}.

The opine synthase genes provide useful tools for studying gene expression in plant cells, because they are constitutively expressed in callus cells as well as in all tissues of plants regenerated from T-DNA-transformed cells^{20,21}. Of practical importance is the fact that very rapid and sensitive assays for the detection of both nopaline and octopine have been developed^{22,23}. Various attempts to express a number of bacterial, animal and some unrelated plant genes (for example, leghaemoglobin) introduced and integrated into the tobacco genome with the use of Ti-plasmid gene vectors²⁴, have thus far failed (unpublished results of this laboratory), because these foreign genes were not transcribed in the transformed tobacco cells. This lack of expression was presumably due to the fact that the plant transcription machinery could not recognize the transcription signals (promoter sequences) carried by these foreign genes.

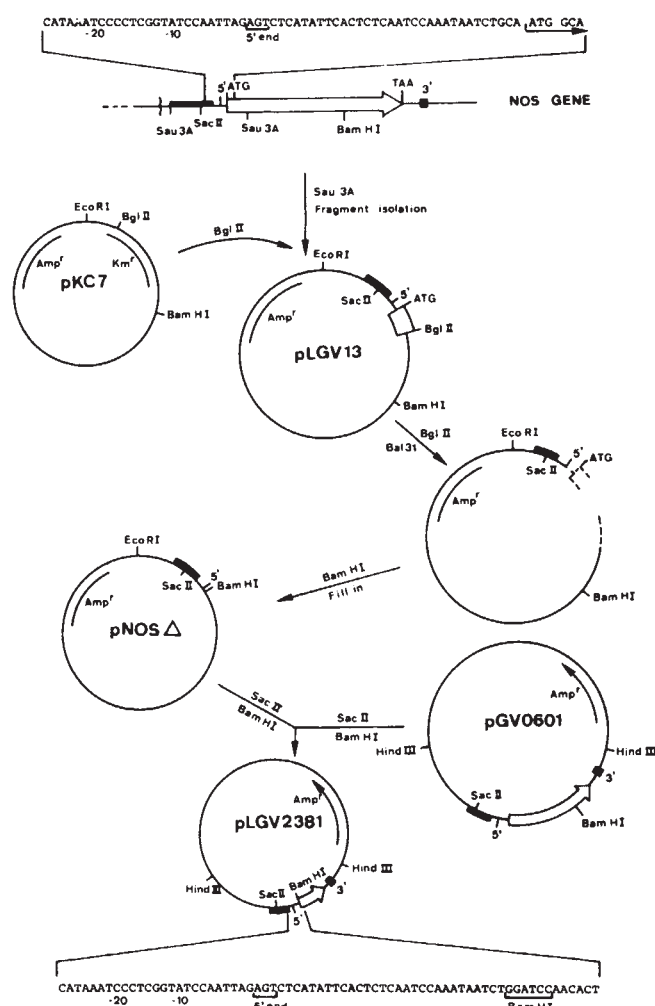


Fig. 1 Construction of a plasmid for expression of genes behind the *nos* promoter. The DNA sequence of the beginning of the *nos* gene is shown at the top of the figure. 10 μ g of pGV0601 (a pBR322 derivative lacking the *Bam*HI site and carrying the *Hind*III-23 fragment which contains the complete *nos* gene)²⁴ were digested with *Sau*3A and the 350-bp fragment carrying the *nos* promoter was isolated from a preparative 5% polyacrylamide gel. The promoter fragment was ligated to *Bgl*II-cut pKC7 (ref. 25), previously treated with bacterial alkaline phosphatase (BAP). 20 μ g of the resulting plasmid (pLGV13) were digested with *Bgl*II and treated with 7 units of the *Bal*31 exonuclease (Biolabs) for 4–10 min in 400 μ l of 12 mM *MgCl*₂, 12 mM *CaCl*₂, 0.6 M *NaCl*, 1 mM EDTA and 20 mM Tris-HCl, pH 8.0, at 30 °C. During this time ~20–50 bp of DNA were removed. The *Bal*31-treated molecules were digested with *Bam*HI and incubated with DNA polymerase (Klenow fragment) and the 4-deoxynucleotide triphosphates (at 10 mM each) to fill in the ends. Plasmids were screened for a regenerated *Bam*HI site derived from the ligation of a filled-in *Bam*HI end and the end of the *Bal*31 deletion. The sizes of the *Bam*HI-*Sac*II fragment of several candidates were estimated in a 6% urea-polyacrylamide gel, and the nucleotide sequences of the candidates with sizes ranging over 160–190 nucleotides were determined by the method of Maxam and Gilbert⁴². A pNOSΔ clone lacking the *nos*-coding sequence and two nucleotides of the leader sequence was used to substitute the *Sac*II-*Bam*HI fragment in the *nos* gene in pGV0601; the final promoter vector is called pLGV2381. All the recombinant plasmids were selected by transformation of the *E. coli* strain HB101. The bases marked 5' and 3' constitute the start and stop of transcription, and the AUG and TAA refer to the codons used to start and stop translation. The heavy black line indicates the *nos* promoter region, and the white area indicates the *nos*-coding region. *Amp^r*, ampicillin resistance; *Km^r*, kanamycin resistance.

To overcome the potential barrier to the expression of foreign genes in plants, we have constructed a number of chimaeric genes consisting of promoter sequences derived from the *nos* gene and of coding sequences derived from foreign genes. These chimaeric genes were introduced in the genome of tobacco with Ti-plasmid vectors and the resulting transformed tobacco cells were shown to express these chimaeric genes and to produce functional proteins.

Expression vector

Figure 1 outlines the construction of an expression vector using the *nos* promoter region. Fragment *Hind*III-23 from plasmid pTiC58 carries the complete coding sequence and the sequences responsible for faithful expression of the *nos* gene in plant cells (ref. 15 and C. Konz *et al.*, in preparation). DNA from pGV0601 (ref. 24) (a pBR322 derivative lacking the *Bam*HI site and carrying the fragment *Hind*III-23) was digested with *Sau*3A and a fragment, carrying the transcription signals and the sequences coding for the first 15 amino acids of the *nos* gene, was subcloned into the *Bgl*II site of the cloning vector pKC7 (ref. 25), resulting—because of the specific sequences involved—in the regeneration of a *Bgl*II site near the initiation codon of *nos* (pLGV13).

Eukaryotic ribosomes seem to use preferentially the AUG most proximal to the 5' end of a messenger RNA to initiate translation^{26,27}. Therefore, pLGV13 was digested with *Bgl*II and treated with the exonuclease *Bal*31 to delete the *nos*-coding sequence remaining in the plasmid. To place a *Bam*HI site at the end of the *Bal*31 deletions, the treated plasmid was digested with *Bam*HI, and the protruding ends were filled in with DNA polymerase. Self-ligation of these molecules followed by screening for the presence of a *Bam*HI-ended *nos* promoter sequences. Three of these, containing *Sac*II-*Bam*HI fragments of sizes 150–180 base pairs (bp), were chosen and subjected to nucleotide sequence analysis, and shown to have 3' ends in different positions of the 5'-untranslated sequence of the *nos* gene. A candidate with a deletion removing the *nos*-coding sequence and two nucleotides of the untranslated 5' leader was chosen for further work (Fig. 1).

The *Sac*II-*Bam*HI fragment from this pNOSΔ plasmid was subsequently cloned back into the plasmid pGV0601 (ref. 24), resulting in a vector which has a *Bam*HI restriction site directly behind the *nos* promoter. This vector contains a deletion of 800 bp of the *nos*-coding sequence, removing 300 of the 400 amino acids of the *nos* protein while retaining the putative signal for polyadenylation and termination of transcription.

An *ocs* 'cassette' fragment

To determine whether the constructed expression vector (pGV2381) would promote expression of coding sequences of other genes, we chose to combine it first with the coding sequence of the octopine synthase gene. Indeed, previous work had established that this enzyme is stable in cells of different plants, and that its activity can readily be assayed; moreover *ocs* activity has never been found to be present in extracts of any of the untransformed plants tested so far²². We therefore constructed a 'cassette' fragment consisting primarily of the coding sequence of the octopine synthase, in such a way that this fragment would be convenient for insertion between this and other potential promoter sequences.

The construction of the octopine cassette is outlined in Fig. 2. A *Bam*HI-*Sma*I fragment from pTiB6S3 carrying the complete coding sequence and part of the promoter of the *ocs* gene¹⁴ was cloned into pBR322 cut with *Bam*HI and *Pvu*II. The resulting plasmid, pAGV828, was digested with *Bam*HI and treated with the exonuclease *Bal*31 to remove the 5'-upstream flanking sequences from the structural part of the *ocs* gene, then digested with *Hind*III, filled in and self-ligated. A set of plasmids were obtained carrying the complete coding sequences of the *ocs* gene with different deletion end points in the 5'-nontranslated leader sequence. To bracket the *ocs*-

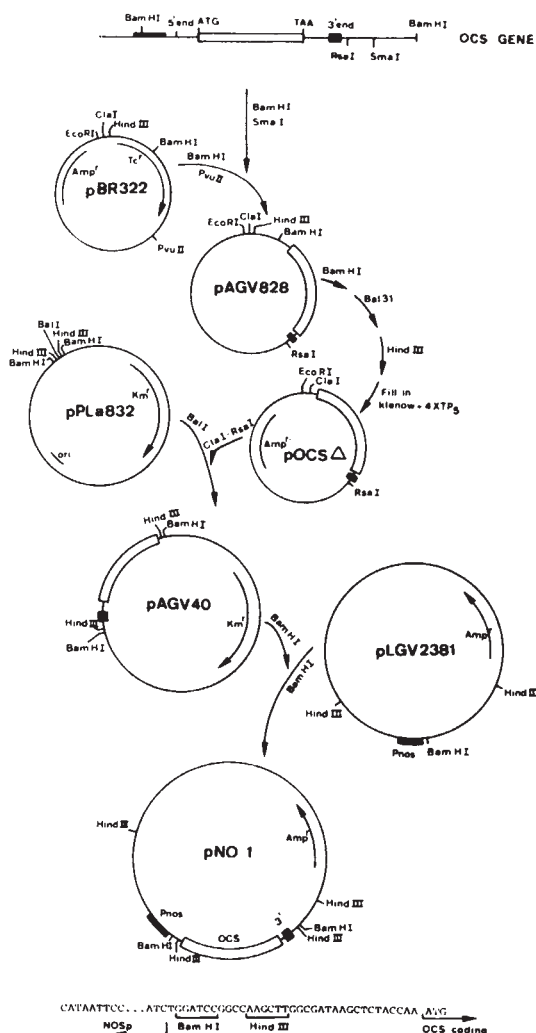


Fig. 2 Construction of a cassette containing the complete *ocs*-coding sequence, and its insertion in plasmid pLGV2381 behind the *nos* promoter. 10 μ g of *Bam*HI fragment 17a of the octopine Ti-plasmid B6S3 were digested with *Bam*HI and *Sma*I, the fragment containing the *ocs*-coding sequence was isolated from a 1% agarose gel, and ligated to the large *Bam*HI-*Pvu*II fragment of pBR322; 20 μ g of the resulting plasmid, pAGV828, were digested with *Bam*HI, treated with the exonuclease *Bal*31 as described in Fig. 1 legend, subsequently digested with *Hind*III, and the ends were filled-in and self-ligated. The sizes of the *Bal*31 deletions were estimated in a 6% polyacrylamide gel. The nucleotide sequences of several candidates were determined, and a candidate having only 7 bp remaining of the 5'-untranslated leader sequence was chosen for further work. To bracket the *ocs* sequence with *Bam*HI sites, the *Cla*I-*Rsa*I fragment was filled in and subcloned into the *Bal*I site of pPLa832. The resulting plasmid pAGV40 was digested with *Bam*HI, the fragment carrying the *ocs* sequence isolated by electroelution from a preparative 1% agarose gel, and ligated to pLGV2381 previously cut with *Bam*HI, and treated with bacterial alkaline phosphatase (BAP). The insertion of the *ocs* sequence in pLGV2381 was obtained in both orientations (pNO1 and pNO2). The junction sequence between the *ocs*-coding sequence and the *nos* promoter in pNO1 is shown. The heavy black line refers to the *ocs* promoter region and the white area to the *ocs*-coding region. Other notations are as for Fig. 1.

coding sequence with *Bam*HI sites, the *Cla*I-*Rsa*I fragment of the resulting pOCSΔ plasmids was isolated, the *Cla*I site was filled in with DNA polymerase and the fragment was cloned into the *Bal*I site of pPLa832 (ref. 28). One of these plasmids (pAGV40) containing only 7 nucleotides of the 5'-untranslated leader sequence and the complete coding sequence, as well as the 3'-untranslated sequence, including the AATAAA signal from the *ocs* gene, was chosen for further work.

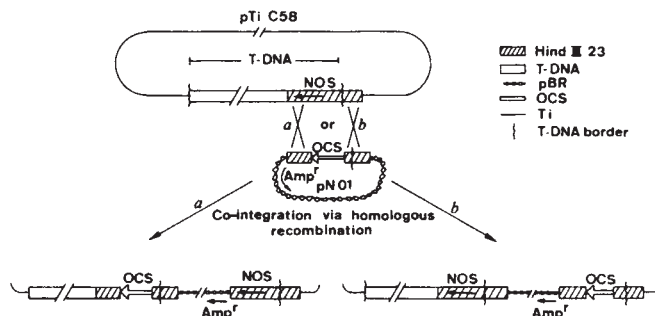


Fig. 3 Possible structure of the co-integrates between pTiC58 and pNO1 in *Agrobacterium*. The helper plasmids that provide the transfer and mobilization functions (R64drd11 and pJG28, respectively)²⁹ were transferred to *E. coli* HB101 (pNO1) by conjugation. The resulting strain carrying all three plasmids were conjugated to *Agrobacterium* (pTiC58) for 16 h on solid Luria broth (LB) medium. Exconjugants carrying the co-integrate pNO1::pTiC58 were selected by plating on LB medium containing 100 μ g ml⁻¹ of ampicillin. The structure of the co-integrates was determined by Southern blotting analysis (data not shown), and the two possibilities for co-integration via homologous recombination between the Ti-plasmid and pNO1 to the 1-kb regions of *Hind*III-23 present in pNO1 is shown.

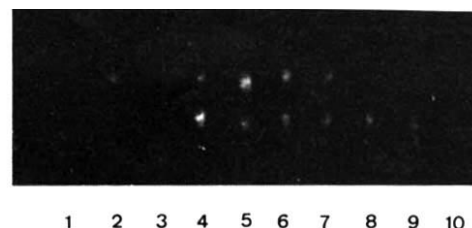


Fig. 4 Detection of octopine in plant tissue transformed with the chimaeric *nos-ocs* gene. Axenic tissue derived from tumours induced with the chimaeric genes were obtained by cutting the tumour into small pieces and subsequently growing *in vitro* on hormone-free Murashige and Skoog (MS) medium in the presence of the antibiotic HR-756 (Hoechst) for several weeks, and then transferring to medium without antibiotic²⁴. 10 mg of the bacterium-free tissue was incubated for 12 h in liquid MS medium containing 0.1 M arginine, ground with a glass rod and centrifuged for 3 min at 12,000 r.p.m. 3 μ l of the supernatant were spotted onto 3 MM Whatman paper and subjected to electrophoresis²². Lane 1 shows the control octopine and nopaline (3 μ l from a solution of 1 mg ml⁻¹ each), and extracts obtained from tissues infected with *Agrobacterium* carrying pTiB6S3 (lane 2), pTiC58 (lane 3), co-integrates pNO1::pTiC58 with structure-type a (lanes 4, 5), co-integrates pNO1::pTiC58 structure-type b (lanes 6, 7), and co-integrates pNO2::pTiC58 (lanes 8-10). Lanes 1 and 3-10 show the presence of nopaline, and lanes 2 and 4-7 show the presence of octopine.

A chimaeric *nos-ocs* gene

A chimaeric gene linking the *nos* promoter sequence to the coding sequence of the octopine synthase gene was constructed as shown in Fig. 2. The *Bam*HI fragment of pAGV40 was cloned into the *Bam*HI site of pLGV2381 in both orientations pNO1 and pNO2, respectively (Fig. 2).

Plasmids pNO1 and pNO2 were transferred by conjugation to *Agrobacterium* and recombined with an acceptor pTiC58 plasmid by a method that allows direct mobilization of pBR322 derivatives or any other ColE1-like plasmid to *Agrobacterium*²⁹. As the origin of replication of pBR322 is not functional in *Agrobacterium*³⁰, all the exconjugants carrying the *Amp*^r marker are derived from homologous recombination between pNO1 and pTiC58. The structure of such a co-integrate is illustrated in Fig. 3. The co-integration of pNO1 and the Ti-plasmid results in a plasmid having two right T-DNA borders³¹ in tandem, due to a duplication of the part of *Hind*III-23 carried by pNO1. Southern blotting, and DNA hybridization analysis (data not shown) of eight independent co-integrates

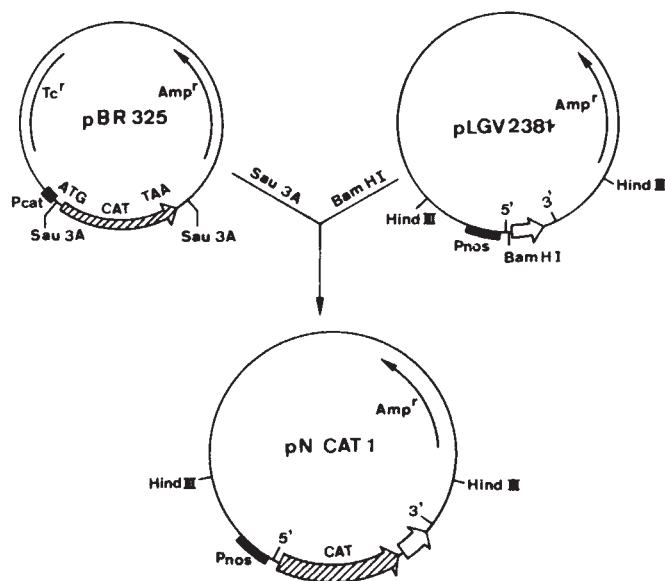


Fig. 5 Construction of a chimaeric gene that directs the expression of the chloramphenicol acetyltransferase in plant cells. 20 μ g of pBR325 were digested with *Sau*3A and the fragment containing the complete coding sequence of the *cat* gene was isolated by electroelution from a 2% agarose gel. The isolated fragment was cloned into the *Bam*HI site of pLGV2381, previously treated with BAP. The orientation of the insert was determined by *Eco*RI and *Hind*III digestion. pNCAT1 carries the *cat* gene in the proper orientation to be transcribed from the *nos* promoter, and pNCAT2 carries the gene in opposite orientation. The small blackened box represents the *cat* promoter, the long blackened box the *nos* promoter, and the cross-hatched box represents the structural part of the *cat* gene.

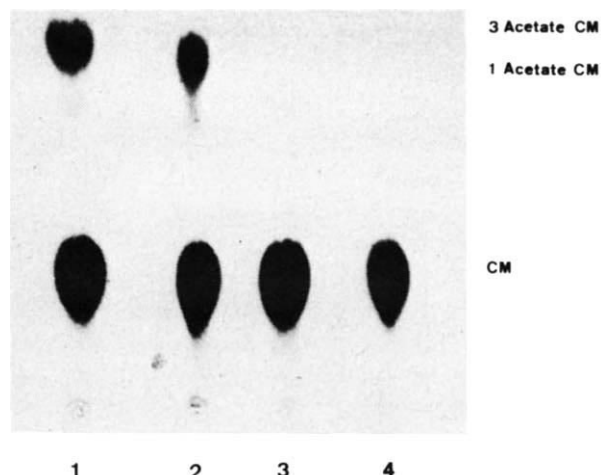


Fig. 6 Assay of *cat* activity in plant cells. 100 mg of tumour tissue was extracted by grinding manually with a glass rod in the presence of an equal volume of buffer containing 0.5 M sucrose, 0.25 M Tris-HCl, 1% ascorbic acid, 0.5 mM leupeptine (Sigma), 10 mM EDTA, 1% cysteine-HCl, pH 7.5, 0.5 mM acetyl CoA and 1 μ Ci 14 C-chloramphenicol (50 μ Ci mmol^{-1} , NEN), and the mix was incubated at 37°C for 30 min (ref. 34), followed by centrifugation (5 min at 12,000 r.p.m.) and removal of the supernatant for analysis. A sonicated extract of *E. coli* containing pBR325 (ref. 32) was used as a positive control. Plant and bacterial reaction mixes were extracted with ethyl acetate, concentrated by evaporation, and subjected to ascending chromatography in a silica gel thin-layer plate with chloroform/methanol (95:5) as eluant. The autoradiogram shown was exposed for 48 h at room temperature. Lane 1 shows the bacterial enzyme control, lane 2 shows the *cat* activity found in cells transformed with *Agrobacterium* containing pNCAT1::pTiC58. No *cat* activity was found in tissue transformed with the strain carrying pNCAT2::pTiC58 (lane 3), even after much longer exposure (data not shown). Lane 4 shows the reconstruction experiment where 10^9 *agrobacteria* carrying pTiC58::pNCAT1 were added to nopaline tumour tissue (induced by *agrobacteria* carrying wild-type pTiC58) before extraction.

revealed that five had the orientation *ocs*-border-*nos*-border (a), and three the orientation *nos*-border-*ocs*-border (b).

Tumours were induced by infecting decapitated tobacco seedlings with eight independently isolated *Agrobacterium* strains carrying the co-integrates pNO1::pTiC58, and four carrying co-integrates pNO2::pTiC58. Tumours were subsequently cultivated *in vitro* in hormone-free medium²⁴ and 10 mg of bacterium-free tissue were used to test for the presence of octopine and nopaline. As shown in Fig. 4, octopine was present in all the tumours induced with strains carrying the co-integrate pNO1::pTiC58 in amounts slightly higher than those found in tumours induced by the octopine wild-type Ti-plasmid pTiB6S3. Furthermore, the presence of both nopaline and octopine in tumours transformed with the pNO1::pTiC58 co-integrates of either type a or type b (see Fig. 3) suggests that both genes are transferred in spite of their position on either side of the internal right border of the T-DNA. This might also suggest that either the most external borders are preferentially used to transfer the DNA positioned between them, or that the presence of two borders flanking any DNA sequence may create a new 'T-DNA' that is independently transferred to the plant genome. As the tumour tissue (used for these experiments) was not cloned, the results may reflect several independent integration events, and further experiments are needed to clarify the structure of the T-DNA present in tumour tissue transformed with the co-integrates used in this study. On the other hand, tumours induced with pNO2::pTiC58, which contains the octopine synthase in opposite orientation to the transcription direction of the *nos* promoter, while positive for nopaline, did not contain octopine.

These results indicate that the *Hind*III-*Bam*HI fragment of pLGV2381 contains a functional *nos* promoter able to promote transcription of the octopine synthase coding sequence, and, therefore, presumably of any other coding sequence properly oriented downstream from this region.

A chimaeric *nos*-*cat* gene

To test further the applicability of the constructed expression vector, the *nos* promoter was combined with the coding sequence of chloramphenicol acetyltransferase (EC 2.3.1.28) (*cat*) from pBR325 (ref. 32). This enzyme of bacterial origin provides a very sensitive and simple assay system, and no similar endogenous activity is present in plant cells. Furthermore, this enzyme has been shown to be expressed in yeast and mammalian cells^{33,34}.

A *Sau*3A fragment from pBR325, containing the complete coding sequence of the *cat* gene, and 56 bp of the untranslated leader sequence where no other ATG than the initiation codon is present^{35,36}, was cloned in two orientations into the *Bam*HI site of pLGV2381 as outlined in Fig. 5. Both pNCAT1 and pNCAT2 (opposite orientation) were mobilized from *Escherichia coli* to *Agrobacterium* as described for pNO1 (Fig. 2), and exconjugants containing pTiC58::pNCAT1 and pTiC58::pNCAT2 co-integrates were selected on ampicillin-containing media. Southern blotting DNA hybridizations (data not shown) demonstrated that strain GVCAT1 contained a pTiC58::pNCAT1 co-integrate, and strain GVCAT2 a pTiC58::pNCAT2 co-integrate, which both resulted from a cross-over similar to situation a illustrated in Fig. 3.

Strains GVCAT1 and GVCAT2 were used to induce tumours on tobacco seedlings and the resulting tumour tissues were grown in axenic cultures²⁴. Extracts from such axenic tissue cultures were tested for *cat* activity. As shown in Fig. 6, the tissue derived from tumours induced by the strain GVCAT1, whose T-DNA contains the *cat* gene-coding sequence properly oriented relative to the *nos* promoter, did indeed contain *cat* activity, as seen by the presence of the same chloramphenicol derivatives as in the control incubated with an extract of *E. coli*

(pBR325). The tumour line induced with GVCAT2, whose *cat* gene-coding sequence is in the wrong orientation, was negative. The fact that tumours induced by GVCAT1 were shown to contain nopaline (data not shown) as well as the observed *cat* activity, suggests that the external border was involved in the integration of the T-region of the co-integrate pTiC58::pNCAT1 in the plant DNA, as discussed above for the *nos-ocs* chimaeric gene.

Agrobacterium strains have been found to contain some endogenous *cat* activity³⁷. To ensure that bacterial *cat* activity cannot account for the observed activity in tobacco tumours induced by GVCAT1, we took particular care to demonstrate that the tumour lines were completely devoid of any surviving bacteria before extraction. Furthermore, wild-type C58 tobacco crown gall tissue was mixed with 10^9 cells from a fresh culture of GVCAT1, and the extraction procedure described in Fig. 6 legend was repeated. No *cat* activity was observed in these circumstances, demonstrating that the very gentle mechanical extraction procedure used to open the plant cells could not lead to a contamination of the plant cell extract with bacterial *cat* activity.

Discussion

The successful expression of the octopine synthase and chloramphenicol acetyltransferase coding sequence as chimaeric genes linked to the transcription-promoting sequence of the nopaline synthase gene demonstrates that this approach can be used to transfer and express the coding sequences of foreign genes in plants. The *nos* promoter is particularly useful because of its wide host range: it is functional in calli derived from all dicotyledonous plants so far tested, and it appears to be expressed in most tissues of regenerated *nos*-containing plants³⁸.

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The easy and sensitive assay for the presence of octopine in plant cell extracts makes the *ocs* cassette a very versatile tool for studying the structure–function relationships of various promoter sequences in plants, especially with regard to their role in the regulation of gene expression and specificity.

Another important application is the construction of general selectable marker genes for plants. Using the *nos* promoter expression vector (pLGV2381), we constructed two such chimaeric genes, one containing the coding sequence of the neomycin phosphotransferase of Tn5 (ref. 39) and the other the methotrexate-resistant dihydrofolate reductase of plasmid R67 (ref. 40). Both these genes were shown to be expressed in tobacco, and able to be used as selectable marker genes⁴¹, thus confirming the general applicability of the constructions reported here. On the basis of these results it can be expected that gene-splicing techniques, so far applied to bacterial and mammalian cells, can be used for plant cells also. Moreover, the availability of simple and rapid techniques for the regeneration of plants from single cells, makes plants an especially interesting system for the study of development and differentiation, as well as for genetic engineering.

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LETTERS TO NATURE

Is the millisecond pulsar formed from coalescence of a close neutron-star binary?

H. F. Henrichs & E. P. J. van den Heuvel

Astronomical Institute, University of Amsterdam, Roetersstraat 15, 1018 WB, Amsterdam, The Netherlands

The combination of short pulse period and abnormally weak surface dipole magnetic-field strength ($B_s \sim 6 \times 10^8$ G (refs 1, 2), in contrast to the canonical pulsar value of $\geq 10^{12}$ G (ref. 3)) of the 1.5-ms pulsar PSR1937+214 (refs 4, 5) has led to the suggestion that it is an old neutron star that was spun up by accretion in a binary system^{6–10}. Here we point out difficulties

with such a model: massive binaries are unlikely to live sufficiently long to allow the required amount of spin-up matter ($\geq 0.1 M_\odot$) to be accreted; in low-mass binaries the companion star is still expected to be present. We argue that a likely alternative is: formation by coalescence of two neutron stars that formed a close binary such as PSR1913+16. These (inevitable) events may occur in the galaxy at a rate $\sim 10^{-4} \text{ yr}^{-1}$, and provide a natural explanation of the millisecond rotation period.

PSR1937+214 shares its combination of short pulse period and abnormally weak surface magnetic field strength with the binary radio pulsars with short orbital periods PSR1913+16 ($P_{\text{orb}} = 7 \text{ h}$ 45 min, $P_{\text{pulse}} = 0.059 \text{ s}$) and PSR0655+64 ($P_{\text{orb}} = 24 \text{ h}$ 41 min, $P_{\text{pulse}} = 0.196 \text{ s}$) which both have $B_s \leq 2 \times 10^{10}$ G (refs 10, 11). The content of rotational energy, however, is in the case of PSR1937+214 three, respectively four orders of magnitude higher than in PSR1913+16 and PSR0655+64.

This enormous difference implies that it is not *a priori* obvious that these objects have had a similar evolutionary history.

Spin-up by accretion in a (fairly) massive binary during a common-envelope (CE) phase is viable for explaining the combination of short pulse period, short binary period and weak surface magnetic-field strength of the two binary radio pulsars mentioned above¹⁰⁻¹⁴. In this model the neutron star—which at the onset of the accretion was already several million years old, such that its surface field may have already partly decayed—underwent copious accretion and was spun-up back to an ‘equilibrium’ spin period^{6,15}:

$$P_{\text{eq}} \approx (2.4 \times 10^{-3} \text{ s}) B_9^{6/7} R_6^{15/7} (M/M_\odot)^{-5/7} \left(\frac{\dot{M}}{\dot{M}_{\text{Edd}}} \right)^{-3/7} \quad (1)$$

The value of P_{eq} depends essentially on the magnetic-field strength B_9 (in units of 10^9 G) and the accretion rate $\dot{M}$ (here written as a fraction of the limiting Eddington accretion rate, $\dot{M}_{\text{Edd}} = (1.5 \times 10^{-8} R_6) M_\odot \text{ yr}^{-1}$); M denotes the mass and R_6 the radius (in units of 10^6 cm) of the neutron star.

The very short orbital periods of the two mentioned binary radio pulsar systems show that a CE (spiral-in) phase with large losses of mass and orbital angular momentum must have taken place for them. In the case of PSR1913+16 the companion star itself must, after the spiral-in phase, have collapsed to a neutron star during a supernova event. This is evidenced by the high orbital eccentricity of the system ($e = 0.617$) (ref. 16) which indicates that the system was almost disrupted by the mass ejection in the second supernova^{11,12,17}. (In the case of PSR0655+64 (which has a circular orbit), after the CE phase the companion most probably remained as a white dwarf^{13,14,18}.)

If PSR1937+214 originated from a massive X-ray binary, its evolutionary history must have been similar to the history of PSR1913+16, the only difference being that the system was disrupted in the second supernova explosion. This implies that the companion of PSR1937+214 was originally a massive star, for the following reason. Assuming a mass of $1.4 M_\odot$ for the two neutron stars produced in the system, the exploding star must have had a mass $\geq 4.2 M_\odot$ just before the explosion, because disruption requires that more than one-half of the total mass of the system was explosively ejected^{11,17} (assuming spherically symmetric mass ejection). At that time the exploding star must have been the core (consisting of helium and heavier elements) of the original companion, since the hydrogen-rich envelope was lost during a preceding mass-transfer (CE) phase^{11,13,17,19,20}. This core mass then implies that before the CE phase the companion must have had a mass of at least $\sim 15 M_\odot$ (for details, see ref. 13). During the CE phase the neutron star is embedded in the envelope of its companion and accretion will take place at the maximum possible Eddington rate, that is $\sim 1.5 \times 10^{-8} M_\odot \text{ yr}^{-1}$. The assumption that during phases of rapid mass transfer the actual accretion rate is limited to $\dot{M}_{\text{Edd}}$ is strongly supported by the observation that the pulse period of PSR1913+16 agrees well with its rotation period predicted with equation (1) by substituting $\dot{M} = \dot{M}_{\text{Edd}}$, together with its observed B_9 -value^{6,11,12}. The same holds for PSR1937+21. Although the latter seems encouraging for accretion scenarios, the massive-binary scenario faces the following problem.

The amount of accreted matter m_a required to spin up the neutron star to an equilibrium (limiting) period P is⁶:

$$m_a \approx (0.22 M_\odot) I_{45} \left(\frac{P}{10^{-3} \text{ s}} \right)^{-4/3} (M/M_\odot)^{-2/3} \quad (2)$$

where I_{45} denotes the moment of inertia in units of 10^{45} g cm^2 . (It is assumed here that all the accreted gas moved in with a keplerian period equal to the present spin period P .) Inserting $P = 0.00155 \text{ s}$ one observes that PSR1937+214 must have accreted $\sim 0.12 M_\odot$.

It is here that the problems for the massive-binary scenario arise, because a companion with $M \geq 15 M_\odot$ is not expected to live longer than $\sim 10^7 \text{ yr}$. To accrete as much as $0.12 M_\odot$ during this time, it would be required that the neutron star was

accreting at the maximum possible rate $\dot{M}_{\text{Edd}}$ during the entire lifetime of its companion. Theoretical considerations^{13,21-23} indicate, however, that such a large mass-transfer rate can occur only during a few short-lasting evolutionary stages of the system. This happens when the companion star is nearly filling its Roche lobe and accretion occurs either (1) by beginning Roche-lobe overflow²¹ or (2) from the enhanced stellar wind of the companion^{19,20}, or by a combination of both. Evolutionary calculations²¹⁻²³ for companions in the mass range $M_c \approx 15-30 M_\odot$ show that these stages last no longer than $\sim 10^5 \text{ yr}$. Subsequently, fully developed Roche-lobe overflow ensues, leading to the formation of a common envelope which is subsequently ejected during spiral-in^{13,14,17,19,20}. (Note that the energy for the ejection of the envelope is not provided by accretion, but is drawn from the orbital energy as a consequence of the very large frictional drag²⁰.) The CE phase lasts at most a few times 10^4 yr (refs 19, 20). If after this stage the remaining core of the companion is sufficiently massive ($> 6 M_\odot$) it may be a Wolf-Rayet (WR) (helium-burning) star with a very strong wind for some $5 \times 10^5 \text{ yr}$ (refs 15, 24). If the orbital period is very short during this stage (of the order of hours) the neutron star may then perhaps still keep accreting at the Eddington limit. For $M_c \approx 15-30 M_\odot$ this leads to a maximum duration of the near Eddington-limit accretion phase of $\sim 6 \times 10^5 \text{ yr}$ yielding a maximum possible amount of accretion of $0.009 M_\odot$. For $M_c \geq 30 M_\odot$ the star may become an Of star with a strong wind ($\leq 10^{-5} M_\odot \text{ yr}^{-1}$) already during the last $1-2 \times 10^6 \text{ yr}$ of core-hydrogen burning. Here, however, the large wind velocities ($\geq 2,000 \text{ km s}^{-1}$) limit the accretion rate onto the companion to $\leq 10^{-9} M_\odot \text{ yr}^{-1}$, which again leads to a maximum possible amount of accretion $\leq 0.01 M_\odot$.

We conclude that origin of PSR1937+214 from a massive X-ray binary is unlikely as the maximum possible amount of accretion that can be reached is at least an order of magnitude smaller than required. (We note that the problem of a too small amount of accretion does not occur for the two binary radio pulsars PSR1913+16 and PSR0655+64.)

It has been suggested⁶ that after the CE phase and the disappearance of the (WR) companion the neutron star may still remain surrounded by a massive disk ($\geq 0.1 M_\odot$) which is subsequently slowly accreted in $\geq 10^7 \text{ yr}$. However, it seems questionable whether such an extended massive disk—if it can exist—could survive the supernova explosion of the WR star at only a few solar radii distance, which takes place within $\sim 5 \times 10^5 \text{ yr}$ after formation of the disk.

Spin-up due to accretion from the wind of an intermediate-mass red-giant companion ($M_c \approx 4-8 M_\odot$ (ref. 9)) is also insufficient to explain the short rotation period. Even when such a companion star is almost filling its critical lobe (overflow is not permitted as this would lead to runaway mass transfer and spiral-in) and the wind velocity is taken to be as low as the escape velocity at the surface of the companion star (that is, the most favourable situation in which the accretion rate is at maximum) the captured fraction of the stellar wind will be not higher than²⁵

$$f \approx \frac{\dot{M}_{\text{acc}}}{\dot{M}_c} = d^2 \left(\frac{1+q}{1+q^{-1}} \right)^2 \frac{1}{[1+d(1+q)]^{3/2}} \quad (3)$$

where $d \equiv R_c/2a$, R_c is the radius of the companion star with mass M_c and mass-loss rate $\dot{M}_c$, a is the binary separation and $q \equiv M_{\text{ns}}/M_c$. Adopting $M_{\text{ns}} = 1.4 M_\odot$ and $M_c = 4-8 M_\odot$, the fraction is $f = 0.0046-0.0015$. However, the mass of the red giant should be $\geq 4.2 M_\odot$ to have disruption of the system in the (final) supernova. This means that at most an amount $f(M_c - 4.2 M_\odot) \approx 0.005 M_\odot$ can have been captured by the neutron star during the accretion phase. As this is 20 times too low to account for the spin period of 1.5 ms we conclude that the origin from a wide intermediate-mass binary is unlikely.

An alternative model^{6,8} is spin-up by accretion in a low-mass X-ray binary. All evidence suggests that the bulge X-ray sources and globular cluster X-ray sources are neutron stars in close binaries which accrete matter from a low-mass companion star

that overflows its Roche-lobe^{26,27}. The shortest orbital period recorded so far is 41 min, for the 7-s X-ray pulsar 4U1626-67. Such periods imply very low companion masses of the order of $\leq 0.1 M_{\odot}$ (if the stars are assumed to be hydrogen-rich). In such close systems mass transfer must be driven by angular momentum losses by gravitational radiation (GR) and, possibly, by additional mechanisms²⁸. What will be the fate of such systems? Theoretical considerations indicate that they will finally spiral-out instead of spiral-in^{29,30}, the simple reason being that the companion becomes either a fully convective red dwarf or a degenerate star. In either case the stellar structure is essentially that of a polytrope of index 1.5, which implies that the radius increases when the star loses mass. During this spiralling out the time scale τ_{GR} for GR losses increases and the rate of mass transfer decreases and finally drops to zero^{29,30}. So the X-ray source will gradually fade away and the companion star is not expected to be fully consumed. (It cannot be torn apart by tidal forces as its dynamical time scale remains $\geq 10^{7-9}$ times shorter than the time scale for orbital decay, even if angular-momentum loss mechanisms several orders of magnitude more efficient than GR would be operating.) Adopting a hydrogen-rich companion and $\tau_{GR} \sim \tau_{Hubble}$ in the final stage, one derives a final orbital period ~ 2.2 h, corresponding to a Roche-lobe filling companion with a mass of $\sim 1.7 \times 10^{-2} M_{\odot}$ and radius $\sim 7 \times 10^4$ km (ref. 31). A $1.4 M_{\odot}$ neutron star will be induced by such a companion to describe an orbit with a radius of $\sim 8,000$ km. For an observer in the orbital plane this would yield a periodic modulation of the pulse arrival times with a double amplitude of 34 pulse periods. The observed limits^{1,2} set on the variation of the pulse period seem to rule out the presence of such a companion star, unless the inclination of the orbital plane is between 88° and 90° (which has a statistical probability of $\leq 10^{-3}$).

Finally we consider the coalescence of a close neutron star-neutron star binary. As mentioned above all available evidence indicates that the binary radio pulsar PSR1913+16 consists of two neutron stars both with a mass of $\sim 1.4 M_{\odot}$. The orbit is observed to decay precisely as predicted by the emission of gravitational radiation according to general relativity¹⁶. This will lead inevitably to a coalescence for this system within $\sim 3.1 \times 10^8$ yr (refs 32, 33). Clark and Eardley³³ have studied the latest stages of orbital decay and coalescence of such a system. They found that: (1) if both components have nearly equal masses they will directly coalesce when their orbital radius becomes < 30 km; (2) if they slightly differ in mass the lower-mass component will first overflow its Roche lobe, resulting in a runaway mass stripping with a peak rate of mass transfer, a brief spiral out followed by complete tidal disruption of the lower-mass star (when its mass drops below the lower mass limit for a stable neutron star, its radius suddenly increases by a large factor). In the latter case a sizeable fraction of the mass of the secondary may be ejected from the system and the remaining star may still be a neutron star³³. Whether in the equal-mass case also a neutron star may remain, depends on the initial masses and on the equation of state of neutronized matter. The orbital period near coalescence is ~ 1 ms and the two (magnetized) neutron stars are expected to be co-rotating. During coalescence a fraction of the orbital angular momentum is radiated away in a burst of gravitational radiation with a strength of 2×10^{52} erg lasting ~ 1 ms. After coalescence, however, the (orbital) angular momentum left is still sufficient for the remaining neutron star to rotate with a period around 1 ms. At rotation periods below ~ 1.5 ms, however, a neutron star is expected to be unstable to the radiation of gravitational waves by non-radial stellar modes³⁴. As a result its rotation period will never drop below ~ 1.5 ms. Hence, the neutron stars that result from the coalescence of a close neutron star binary are always expected to be found with a rotation period ≥ 1.5 ms. It can hardly be a coincidence that this is just the rotation period of PSR1937+214. Hence, whereas origin by spin-up in a massive binary system faces difficulties in providing the necessary amount of accretion—and thus, of rotational energy

and angular momentum—the coalescence of two orbiting neutron stars gives the correct order of magnitude for these quantities. (The details of the coalescence, however, may be subject to more study.)

It has been shown³⁵ that the galactic formation rate of close neutron-star binaries, as evolutionary products of persistent massive X-ray binaries, may be as high as about $3 \times 10^{-4} \text{ yr}^{-1}$. We do not know precisely with what orbital periods neutron-star binaries are born. The observed post-spiral-in period 0.63 h (ref. 36) of the X-ray pulsar 1E2259+586 (which still is surrounded by a large ejected shell³⁷) and 7 h 45 min of PSR1913+16, suggest formation with periods of the order of ~ 1 –8 h. Systems with $M_1 = M_2 = M_{\odot}$ and a period ~ 6 h and $e \approx 0.8$ (or 1.6 h and $e \approx 0$) will coalesce within 5×10^7 yr after formation³². Assuming a decay time scale of $\sim 5 \times 10^6$ yr for neutron-star crustal magnetic fields^{3,38}, an original surface magnetic field of $\sim 5 \times 10^{12}$ G will have decayed to $\sim 6 \times 10^8$ G in this time. (The newly formed neutron star may be hot, but this will not necessarily lead to the generation of a strong surface field by thermal effects³⁹, as it is not known whether the conditions required for this process to operate—a relatively long electron collision time or the presence of a surface layer of helium—are fulfilled here.) Hence, PSR1937+214 may have formed from a system very similar to PSR1913+16.

Unfortunately, we do not know what fraction of the coalescing systems will finish as neutron stars and what fraction collapses to a black hole. We note, however, that even if only 1% of the close neutron-star binaries coalesces to neutron stars that are observable as a weak magnetic-field pulsar, the galactic formation rate of millisecond pulsars may still be as high as $\sim 3 \times 10^{-6} \text{ yr}^{-1}$. With a lifetime as an observable pulsar of $\sim 10^7$ yr (ref. 3), there should then at least be ~ 30 such pulsars in the galaxy. Consequently, even if only 1% of the binary neutron stars formed in the galaxy ends up as objects like PSR1937+214 the presence of one such object within 3–5 kpc of the Sun is not surprising. Also, the position not far from the galactic plane is understandable, as the same is true for the progenitors: binary radio pulsars and massive X-ray binaries.

We conclude that a most likely formation mechanism of PSR1937+214 is coalescence of a close neutron star binary since: (1) we know the existence of a progenitor system in which such a process is inevitable; (2) this provides the millisecond rotation period and possibly also the weak surface magnetic field; and (3) such a process is expected to occur sufficiently frequently in the galaxy to provide a sizeable formation rate of millisecond pulsars.

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Note added in proof: Arons⁹ has independently concluded that origin of the millisecond pulsar by accretion in a massive or a low-mass X-ray binary is highly unlikely.

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Direct observational upper limit to gravitational radiation from millisecond pulsar PSR1937+214

J. Hough, R. W. P. Drever*, H. Ward, A. J. Munley, G. P. Newton, B. J. Meers, S. Hoggan & G. A. Kerr

Department of Natural Philosophy, Glasgow University, Glasgow G12 8QQ, UK, and *California Institute of Technology, Pasadena, California 91125, USA

The newly discovered millisecond pulsar PSR1937+214 has such a high rotational rate, 642 Hz, that any significant quadrupole moment not aligned with the spin axis could lead to a large flux of gravitational radiation. Although recently reported^{1–3} slowdown rates suggest an energy loss corresponding to a currently unobservable level of gravitational radiation, existing evidence may not completely rule out all possibilities for production of detectable gravitational waves. For example, continuing accretion might provide additional energy for gravitational radiation; and it may not be impossible that the pulsar might be closer to the Earth than indicated by the dispersion measure if there was large local dispersion. Overall, the parameters of this pulsar seem so exceptional that a direct search for gravitational radiation with existing equipment seemed worth making. We describe here such a search that has set an upper limit to gravitational wave amplitude at twice the pulsar rotation frequency, the observed output from a gravitational wave detector corresponding at this frequency to an amplitude of $(0.8^{+1.5}_{-0.6}) \times 10^{-20}$.

The most sensitive immediately available instruments for this work were a pair of divided bar gravitational wave detectors developed originally at Glasgow for searches for gravitational wave pulses⁴ and for a stochastic gravitational wave background⁵. These detectors were designed to have a relatively wide bandwidth (several hundred hertz), which happens to include at near maximum sensitivity the frequency 1,284 Hz, twice the repetition frequency of radio pulses from the pulsar. This is a probable frequency for gravitational wave generation, and this search was centred there. The detector used operates at room temperature, with motion sensing by piezoelectric transducers mounted between two half-bars giving a mass of 300 kg, a coupling coefficient of 0.18, a mechanical quality factor of 2,200, and an overall noise temperature near 5 K for detection of millisecond pulses and less than this with appropriate filtering for searches for continuous radiation.

In the present work the relevant part of the spectrum of the detector output, compensated for the changing Doppler shifts due to the Earth's motion, was analysed over an 8-h run with frequency resolution of the order of 3 parts in 10^8 . To reduce

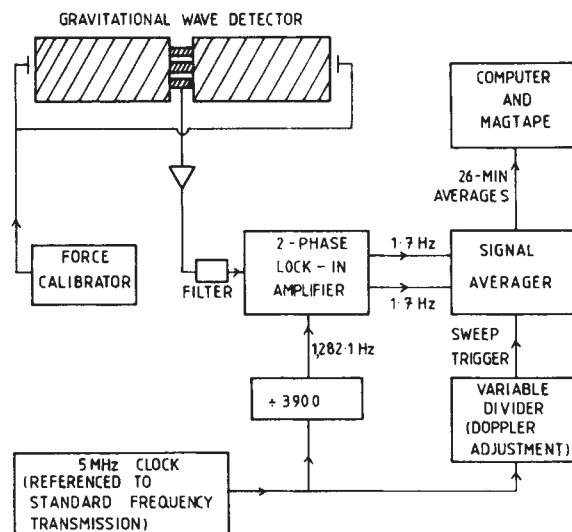


Fig. 1 Simplified schematic diagram of detector and data recording system.

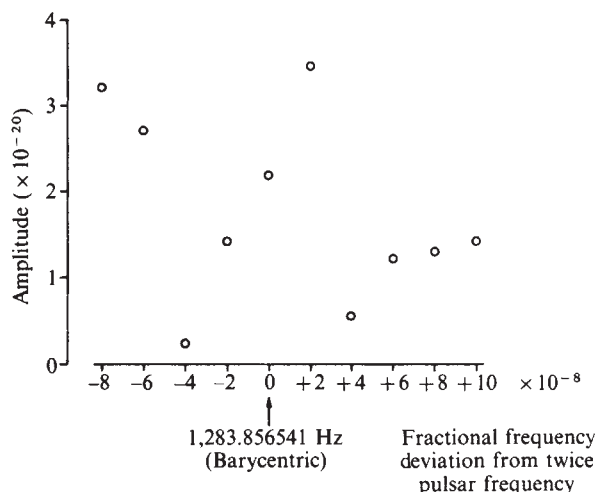


Fig. 2 Analysis of detector output from 8-h run, with output voltage components at 10 discrete barycentric frequencies expressed in terms of equivalent gravitational wave amplitude averaged over polarization.

data handling problems the signal, after filtering by bandpass and notch filters⁴, was heterodyned to near 1.7 Hz by beating with a stable reference sinusoid at (5/3.9) kHz in a two-phase lock-in amplifier, as indicated in Fig. 1.

The two quadrature components of this signal were averaged in 26-min batches by a digital signal averager whose sweep was triggered at a precise adjustable periodicity near 12.57 s. The trigger timing was derived from a stable clock, referenced to the 60 kHz MSF standard frequency transmission, by a system of preset scalars operating in 'leap-year' mode⁶. Small adjustments to trigger period were made throughout the run to take account of changes in apparent pulsar period due to the Earth's motion, and keep relative phase errors less than 0.1 cycle at all times. The 26-min averages were read out to magnetic tape by a small computer, and these data were subsequently combined with appropriate phase shifts introduced between batches to allow searches for a pulsar signal over 10 adjacent frequency channels around the expected frequency. The amplitudes of the frequency components were obtained by folding and least square fitting. Detector sensitivity was calibrated by applying known sinusoidal forces to the ends of the bars by electrostatic plates⁴, and the accuracy of timing and phase of the whole system was monitored by auxiliary counting equipment (not shown).

The resulting frequency analysis of the output from the detector over an 8-h run between 20.15 6 December and 04.15 7 December 1982 is shown in Fig. 2. Here the amplitude of observed output at each of the discrete frequencies indicated is given in terms of the corresponding r.m.s. gravitational wave amplitude. The vertical scale is the amplitude averaged over polarization, taking into account the varying sensitivity of the detector arising from the changing angle throughout the run between the pulsar direction and the axis of the detector, which was aligned north-south. We see no sign of a signal at twice the pulsar frequency. At this frequency the detector output power, corrected for noise power estimated from the data at the other frequencies, corresponds to a gravitational wave amplitude averaged over polarization of $(0.8^{+1.5}_{-0.8}) \times 10^{-20}$. Furthermore, over the whole frequency range investigated, extending to ± 0.13 mHz from twice the pulsar frequency, there is no statistically significant evidence for any narrowband signal.

The negative result found here is not surprising, particularly in view of the limited sensitivity of the available detector. It can be expected that much higher sensitivity will be obtained with tuned cryogenic bars and with laser interferometer gravitational wave detectors under development; as the latter, in particular, will cover a wide range of frequencies there may be possibilities for detection of signals from other fast pulsars. Meanwhile, the present limit provides a direct observational constraint for pulsar PSR1937+214.

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Inversion symmetry of radio lobes in extragalactic sources

Gopal-Krishna & S. M. Chitre*

Radio Astronomy Centre, Tata Institute of Fundamental Research, PO Box 1234, Bangalore 560 012, India

The collimated beams of relativistic particles ejected from the central engines of extragalactic radio sources are traced out by means of the jet-like features of nonthermal radio emission which are observed to extend up to a few hundred kiloparsecs¹⁻⁴. These jets exhibit departures from linearity in a variety of ways. In some less luminous large radio galaxies, the jets show structures with a distinctive S-shaped bending (inversion symmetry) at distances of the order of 100 kpc from the galactic nucleus⁵⁻⁸. We propose here that such a sharp bending can result from interaction of the relativistic beam fluid with faint, rotating shells similar to those seen around some nearby galaxies⁹, and discuss the possible implications of this hypothesis.

Recent observations of many radio jets have revealed marked departures from linearity on a variety of physical scales: for instance, a continuous bending on the very long baseline interferometer scale^{3,10}, an oscillatory jet profile^{11,12}, C-shaped or S-shaped bending on galactic¹³⁻¹⁵ and extragalactic^{3-8,16,17} scales. The mechanisms for explaining such morphologies have been reviewed by Rees *et al.*¹⁸ and Miley². Here we shall be concerned with the large-scale jets from some low-luminosity

radio galaxies which show a pronounced S-shaped bending (inversion symmetry) on the scale of tens to hundreds of kpc. Such radio structures are generally associated with galaxies which are not members of clusters, but which usually have a few galaxies in the neighbourhood¹⁹⁻²¹. The prominent examples⁵⁻⁸ of the large-scale S-shaped morphology include the low-luminosity radio galaxies NGC315, NGC326 and 3C192. It has been suggested that these distortions could result from the precession of the central engine with the twin jets essentially simulating a pair of precessing searchlights^{5,22,23}. A difficulty²⁴ with this model is that if the precessing jet is to remain straight over hundreds of kpc (as seen²⁵ in NGC315), in spite of the ram pressure exerted by the circumgalactic gas, implausibly low gas densities of $\rho \leq 10^{-32}$ g cm⁻³ assuming^{1,26,27} precession rate $\phi \geq 10^{-15}$ s⁻¹ would be required. This also implies a transverse velocity of $\geq 0.1c$ for the precessing jet over most of its length. However, there is no evidence for relatively enhanced radio emission from the side of the jet supposedly subject to the ram pressure⁴. Recently Wirth *et al.*²⁸ have suggested that the gaseous halo of a radioactive elliptical galaxy could become impulsively torqued by tidal effects of a closely interacting galaxy, and the twin jets coupled to the halo would then be bent into an S-shaped morphology. Such a mechanism may operate in some cases, but it is difficult to explain the abrupt bends in otherwise straight jets (as seen²⁵, for example, in NGC315), especially when such bends occur at distances of several hundred kpc, well beyond the extent of any gaseous halo.

We propose here that the S-shaped distortions, on the scales of tens to hundreds of kpc, could arise when the jets impinge on narrow shell or ring-like features surrounding the galaxy. Recently, deep optical photographs of a few nearby ellipticals have revealed^{9,29} the existence of multiple shell segments to distances of a few hundred kpc. Such partial shells may be formed during the merger of a disk galaxy with a massive elliptical²⁹. The computer simulation studies of Quinn²⁹ have shown that a tidal shearing of the captured disk galaxy leads to the formation of rotating multiple shell segments which subtend a substantial solid angle at the nucleus of the elliptical galaxy^{9,29}. The shells are maintained in equilibrium by the gravitational pull of the central elliptical galaxy being countered by the centrifugal force of the rotating shell material. At a typical distance $R_s \sim 100$ kpc from the central galaxy of mass $M_s \sim 10^{12} M_\odot$, the keplerian velocity of the shell material $V_\phi = (GM_s/R_s)^{1/2} \approx 200$ km s⁻¹. Although most of the shell mass is of stellar origin, a small fraction ($\approx 1\%$) of the total gas content of the merging disk galaxy is expected to go into the formation of the shells. Possible evidence for thermal plasma with a density $\rho_s \approx 10^{-28}$ g cm⁻³ in the shells comes from the observed radio depolarization²⁵ in the region where the jet of NGC315 undergoes a sharp bending. A necessary condition for effective bending of the beam is that the relativistic beam fluid is able to penetrate only part way into the shell before being swept downstream by the rotating shell. For a typical shell distance $R_s \sim 100$ kpc, the width⁴ of the jet d_j is usually ~ 5 kpc. Let us denote $\dot{E}_b$ as the rate of energy flowing through the beam and V_b as the bulk velocity of the beam fluid, which is taken to be at least as large as the typical speed ($\sim 0.1c$) of the hot spots in the lobes of radio sources^{2,30,31}. The momentum flux of the beam fluid is then given by $p_b \sim \dot{E}_b/V_b d_j^2$. For an effective deflection of the beam, p_b must be exceeded by the transverse ram pressure exerted by the rotating shell material of density ρ_s , which is given by $p_s \sim \rho_s V_\phi^2$. Thus, the beam bending would occur provided $p_b > p_s$, or $\dot{E}_b < \rho_s V_\phi^2 d_j^2$. Adopting typical parameter values, the condition becomes: $\dot{E}_b \leq 10^{41}$ erg s⁻¹. Note that for a relativistic bulk velocity of the beam fluid, the condition for effective bending would be $\dot{E}_b \leq 10^{42}$ erg s⁻¹. Interestingly, a pronounced large-scale S-shaped morphology is found only in those radio galaxies which are not very luminous and which are preferentially located outside clusters of galaxies^{2,19-21}, their relatively less disturbed environment being more conducive to shell formation²⁹. In some radio galaxies,

* Permanent address: Tata Institute of Fundamental Research, Bombay 400 005, India.

the twin beams may be partly intercepted by the rotating shells, in which case only a portion of the beam fluid will be swept along the shell, while the rest will advance unhindered as, for example, in the double radio galaxy 3C192 with its two bright hot spots located beyond the S-shaped radio arches^{7,8}. Alternatively, the twin beams with low power may be intercepted by a shell creating S-shaped arches and later, because of a boost in the nuclear activity, the beams could become sufficiently powerful to cut through the shell and forge straight ahead.

The typical time scale for sweeping the relativistic fluid across the width of the jet is $\tau = d_j/V_\phi \sim 10^7$ yr. Therefore, the age of the relativistic particles deflected along the shell would normally be of the order of $\geq 10^8$ yr, which is long enough for synchrotron losses to produce a spectral bending at centimetre wavelengths for a magnetic field strength of some few microgauss. Such a spectral behaviour has indeed been observed^{7,25} in the arch-like radio lobes of the galaxies NGC315 and 3C192. Also, the magnetic field in radio arches is expected to be largely aligned in the direction of rotation of the shell material, as a result of the sweeping of the beam fluid along the shell.

Hitherto, we have restricted our discussion to the possibility of beam bending in weaker radio sources ($P_{\text{radio}} \leq 10^{42}$ erg s⁻¹). Recently, large-scale jets have been detected in many high-luminosity radio sources associated with quasars³²⁻³⁴. In view of the high-energy flux in the beams of quasars ($\dot{E}_b \rightarrow 10^{45}$ erg s⁻¹), it is unlikely that they would be effectively arrested. However, the bending of such energetic beams could take place provided the densities in the intercepting structures are a few orders of magnitude higher than the value of $\sim 10^{-28}$ g cm⁻³ which we have adopted for the shells in the earlier discussion. Possibly, gas-rich galaxies are directly associated with many quasars or be physically interacting with them³⁵⁻³⁸. Indeed, the interaction of the beams of some quasars with the spiral arms having densities as high as $\sim 10^{-24}$ g cm⁻³ may well lead to kinks or sharp bends in their jets (like those observed³³, for example, in the quasars 3C196, 3C309.1, 3C380 and 1636+473). The observed S-shaped radio structures in the spiral galaxy NGC4258 and in a few Seyfert galaxies have already indicated the possibility of interaction between the rotating gas disk and the two opposite streams of matter ejected from the galactic nucleus^{39,40}.

Our proposal predicts that a large-scale inversion symmetric morphology would be associated only with those radio galaxies which have low luminosities ($\leq 10^{42}$ erg s⁻¹). The detection of shell-like structures in the deflected portions of the large-scale radio jets would support the mechanism. The shell properties would also provide a valuable diagnostic probe of the physical parameters of the beam fluid dynamically interacting with the shells.

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Interstellar ice grains in the Taurus molecular clouds

D. C. B. Whittet*, M. F. Bode||, A. J. Longmore‡, D. W. T. Baines|| & A. Evans†

* Division of Physics and Astronomy, Preston Polytechnic, Preston PR1 2TQ, UK

† Department of Physics, University of Keele, Staffs ST5 5BG, UK

‡ UK Infrared Telescope Unit, 900 Leilani Street, Hilo, Hawaii 96720, USA

§ Department of Physics and Astronomy, University College, London WC1 6BT, UK

We report here observations made in November 1981 using the United Kingdom Infrared Telescope (UKIRT) at Mauna Kea of the 3 μ m ice absorption feature in the spectra of several obscured stars in the Taurus interstellar clouds. The feature correlated in strength with extinction at visual wavelengths (A_V), and is present in stars with A_V as low as 4-6 mag, a remarkable result when compared with other regions of the Galaxy. Ice may be widespread in the Taurus clouds, vindicating ideas on grain composition and growth first reported nearly 50 yr ago¹.

Ice was first suggested¹ as a constituent of interstellar grains in 1935¹; subsequently, a detailed grain model was developed² based on the nucleation and growth of ice grains in interstellar clouds, a model which achieved widespread acceptance for many years³. However, IR astronomy has demonstrated that ice is an illusive material in interstellar space: the expected spectral feature close to 3 μ m characteristic of water-ice is not detected towards distant stars seen through low density material in our Galaxy^{4,5}, and is apparently restricted to a relatively small number of sources deeply embedded in dense molecular clouds^{6,7}. It is no longer possible to explain all the observed properties of interstellar grains in terms of a single substance. Materials suggested recently as grain constituents have ranged from minerals such as silicates and graphite, to complex organic polymers (see ref. 8 for a review), with the addition of ice in the highest density regions.

Two difficulties have arisen in understanding the location and extent of ice absorption in interstellar clouds. The first concerns the identification: the usual assignment of the 3- μ m absorption feature to O-H stretching vibrations in a solid H₂O matrix has been challenged⁹; however, recent observations¹⁰⁻¹² and associated theoretical and laboratory investigations¹³⁻¹⁵ have tended to confirm an identification with volatile grain material in which water-ice is the principal constituent. The second problem concerns the location of the absorbing region: is the 3- μ m feature characteristic of all molecular clouds in a particular

|| Present addresses: Los Alamos National Laboratory, New Mexico 87545, USA (M.F.B.); Royal Observatory, Edinburgh EH9 3HJ, UK (D.W.T.B.).

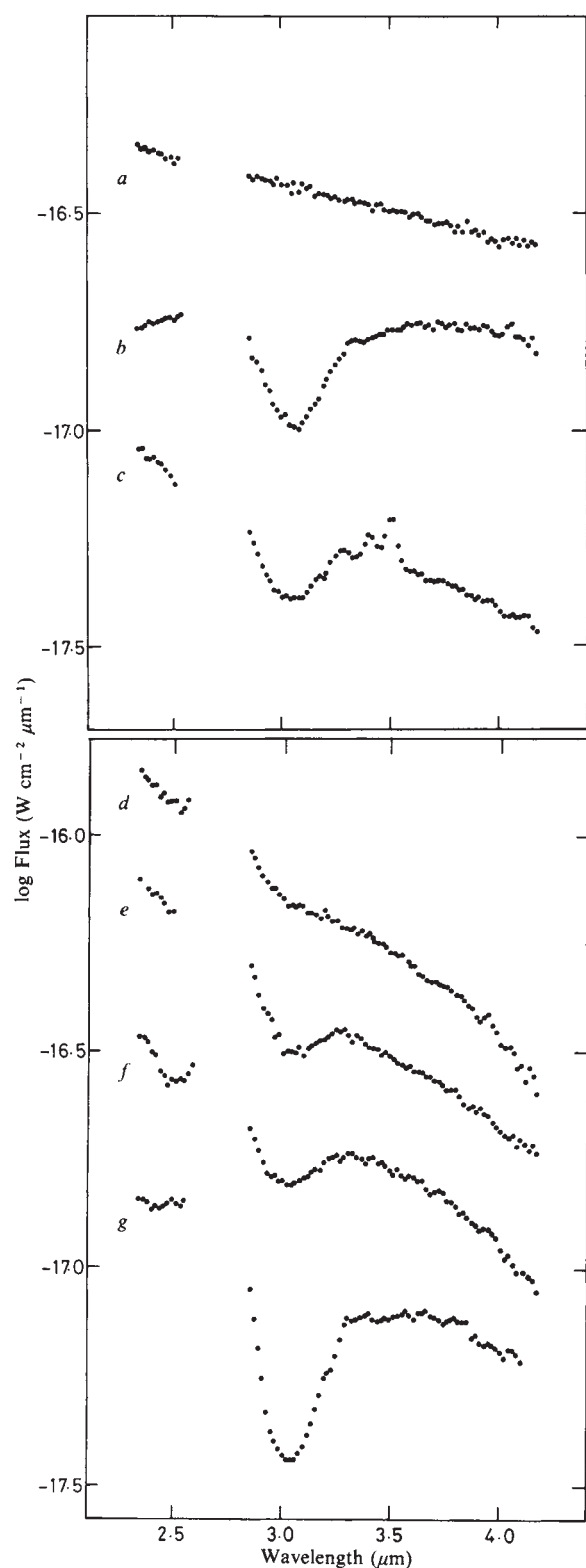


Fig. 1 *a-c*, IR (2.3–4.1 μm) spectra of three dust-embedded stars: *a*, XL Tau; *b*, HL Tau; *c*, Elias 1. The log flux scale refers to the upper spectrum; the others are displaced vertically for display purposes. *d-g*, IR (2.3–4.1 μm) spectra of four reddened field stars: *d*, Elias 9; *e*, Elias 3; *f*, Elias 6; *g*, Elias 16. The log flux scale refers to the upper spectrum; the others are displaced vertically for display purposes.

evolutionary phase, given sufficient shielding from the stellar UV radiation field, or is it restricted to the circumstellar environments of particular protostellar condensations within the clouds? The unexpected absence of the feature in certain lines of sight^{16,17} has led to the suggestion that ice is limited to cocoons around particular protostars, perhaps resembling

Table 1 Observed stars

Star	Spectral type ^{16,18}	Comparison used ^{20,21}	τ_{max} (± 0.08)	A_V ^{16,18} (mag)
Elias 1	Ae + 1,500 K dust	1,500K bb	0.51	8–10
Elias 3	K2III	4,460K bb	0.61	8.1
Elias 6	M8III	M8	0.27	5.9
Elias 9	M4III	M3III	0.10	4.5
Elias 16	K1III	4,580K bb	1.60	20.6
HL Tau	T Tau	1,100K bb	0.85	6–10
XL Tau	T Tau	—	<0.05	3

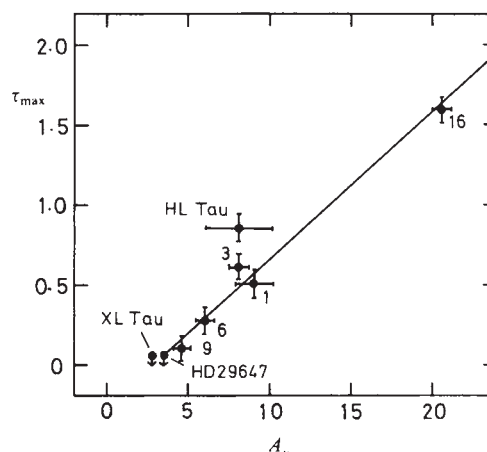


Fig. 2 Plot of peak optical depth (τ_{max}) in the 3- μm feature against visual extinction (A_V). The best straight line for the Elias sources is shown.

primitive Oort cometary clouds. The observations discussed here provide new insight into this problem. The dark clouds in Taurus provide an ideal test case: they form one of the closest regions of continuing star formation to the Solar System¹⁸; however, few luminous OB stars have yet appeared, and the grain material should thus be relatively unmodified by the effects of UV radiation.

The sources observed were mostly selected from the survey of Elias¹⁸ (see Table 1). The observations were made in November, 1981, with a nitrogen-cooled spectrophotometer system at the $f/35$ focus, using an InSb detector and a circular variable filter covering the wavelength range 2.3–4.6 μm at a spectral resolving power of ~ 100 . The useful spectral regions, governed by the atmospheric opacity, are 2.3–2.6 μm and 2.9–4.1 μm . Spectral scans were averaged until a signal-to-noise ratio of at least 20 was achieved. Sky conditions were excellent throughout. Standard unreddened stars were observed at similar airmass: division of source spectrum by standard spectrum removes atmospheric extinction and the instrumental profile, and the spectra were then reduced to relative flux by assigning a black-body curve of appropriate temperature to the standard star. The wavelength scale was calibrated using observations of the emission line sources¹⁹ HD44179 (3.28 μm) and IC418 (3.28 μm and 4.05 μm Br α), and is accurate to ± 0.01 μm . The final spectra, which cover the range 2.3–4.1 μm , are presented in Fig. 1. The accuracy may be judged from the point-to-point fluctuations, and in most cases is comparable with the size of the points.

One previous detection of 3- μm absorption has been reported¹⁶ in the Taurus clouds, that for the T Tauri star HL Tau. We have obtained higher signal-to-noise data for HL Tau, as well as observing other sources. The observations cover two distinct classes of object: dust-embedded stars (HL Tau, XZ Tau and Elias 1: see Fig. 1*a-c*) and background field stars seen through the cloud by chance alignment (Elias nos 3, 6, 9 and 16; see Fig. 1*d-g*). As the latter are all late in spectral type¹⁸, their 2.3–2.5 μm spectra are affected by photospheric CO absorption. A prominent absorption feature centred between 3.0 and 3.1 μm can be seen in stars from both groups. In

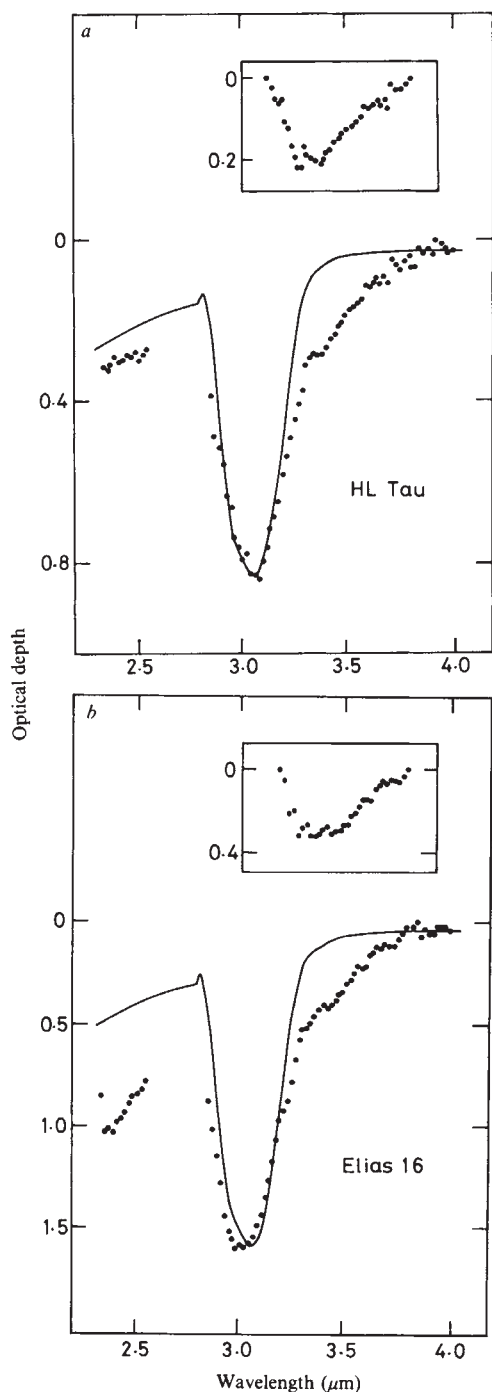


Fig. 3 The observed feature profiles for: *a*, HL Tau and *b*, Elias 16 compared with theoretical modelling for silicate core/amorphous ice mantle grains (curves). The insets show the difference curves, illustrating the long-wavelength wing. The discrepancy between observational data and model at short wavelengths for Elias 16 is due to CO absorption in the stellar atmosphere.

particular, detection of the feature in four sources viewed through the cloud is a significant result, strongly suggesting that the band carrier is widespread and not restricted to the immediate circumstellar environments of particular protostars. The stars in Fig. 1*d-g* are displayed in order of increasing A_V , and a correlation between the strength of the feature and A_V is suggested. This is confirmed by Fig. 2, which shows a plot of the optical depth at the centre of the absorption feature (τ_{max}) against A_V . Values of τ_{max} were deduced using the comparisons listed in Table 1 (see refs 20, 21) to separate the stellar and interstellar components of the spectra. Errors in τ are almost entirely due to uncertainties in the continuum level. Data for

an additional star in the clouds (HD29647) were taken from ref. 22. Figure 2 suggests that a threshold optical depth of ~ 5 mag in A_V is required before the 3- μm feature is of detectable strength. At lower values, the carrier is assumed to be sublimed or photoprocessed by UV radiation. Such a low threshold is unprecedented: in the diffuse interstellar medium^{4,5} and in other dark molecular clouds superficially similar to Taurus, such as those in Ophiuchus^{17,23} and Orion²⁴, the corresponding value of A_V is at least 20 mag. Clearly the Taurus cloud is much more conducive to the survival of the 3- μm band carrier than other clouds which have been studied.

Theoretical extinction curves have been calculated using the Mie theory for spherical core-mantle grains. The core material was assumed to be silicate, with complex refractive index $(1.6 + 0.001i)$ independent of wavelength. The mantle was amorphous water-ice with refractive index values from Leger *et al.*^{15,25}. Best fits to the observed spectrum were obtained with grain cores of radius 0.20 μm and mantles of uniform thickness 0.02 μm . The results are illustrated in Fig. 3*a* for HL Tau and Fig. 3*b* for Elias 16. The extinction is normalized to be zero at the long-wavelength end of the spectrum (4.1 μm) to allow direct comparison between observed profile and model. Figure 3 shows that the observed feature has a long-wavelength wing compared with the model, in common with previous observations⁶⁻⁸. This is illustrated by the inset, which is the difference between the two curves.

The profile of the feature in HL Tau is significantly different from those of Elias 16 and the other Elias sources. In HL Tau (Fig. 3*a*) the observed central wavelength of 3.06 μm agrees well with that of the computed spectrum, and the profile is closely matched over the range 2.9–3.2 μm . Even the slight asymmetry of the main feature is reflected in the model. Hence the HL Tau spectrum appears to be well represented by the model except for the long-wavelength wing. Elias 16 (Fig. 3*b*) is representative of all the Elias sources we have observed to within the observational errors. The mismatch in the 2.3–2.5 μm region is due to stellar CO absorption, which is not allowed for in our comparison spectrum (Table 1). In this case, the central wavelength of the interstellar feature is close to 3.0 μm , slightly shorter than expected, and only in the range 3.05–3.25 μm is a good fit obtained. The long-wavelength wing is present as before (see inset, Fig. 3*b*), but, apparently there is also excess absorption just shortward of the main peak.

The most likely reason for the failure of the model to fit the whole of the profile rests in the assumption that the mantle is composed of pure H₂O ice, producing O–H bond absorption only. The difference curves in Fig. 3 resemble the 3.4- μm absorption feature observed by Allen and Wickramasinghe²⁶ towards the galactic centre, and attributed to organic grain material. We do not attempt modelling with composite materials here, but note that recent investigations^{12–15} have shown an ice mixture, in which H₂O and NH₃ are the main constituents, to be capable of matching the entire profile. Ammonia produces additional absorption at ~ 2.95 μm , and the long-wavelength wing is produced either by scattering due to large grains or by additional impurities, notably molecules containing C–H bonds. The difference in profile we have observed for the Elias sources and HL Tau suggests compositional variations with a depletion of NH₃ towards the latter.

An absorption which resembles the 3- μm interstellar feature when observed at comparable resolution has been detected in the spectra of carbon stars, and attributed, on the basis of high resolution observations²⁷, to circumstellar gas phase molecules such as C₂H₂ and HCN. It is unlikely that the absorption features now reported could be assigned in this way. The long-wavelength wing, which almost always accompanies the ice feature, is not present in the carbon star spectra. Moreover, Elias 1 and HL Tau cannot be carbon stars, and it is very unlikely on statistical grounds that the four background field stars we have observed are all carbon-rich, producing a correlation between τ_{max} and A_V (Fig. 2) by chance. Thus our conclusion that the absorber resides in the interstellar molecular

clouds rather than in circumstellar shells surrounding these field stars holds regardless of its identity. We cannot entirely exclude the possibility that the 3- μm feature is produced by a superposition of gas phase absorptions in the molecular clouds: observations at higher resolution are needed to distinguish unambiguously between gas phase and solid state models.

The spectrum of Elias 1 is notable, as it contains emission peaks at 3.29, 3.42 and 3.52 μm (Fig. 1c). Similar emission features have been detected²⁸ in the spectrum of the Herbig Be star HD97048 and are discussed in detail elsewhere²⁹⁻³¹. They are believed to be the product of solid state resonances in irradiated circumstellar grains and are not therefore directly related to the 3- μm feature, which is never seen in emission⁶.

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An identification of the 3.4 and 3.5 μm features with H_2CO in grain mantles has been proposed elsewhere^{28,31}.

Observations of higher spectral resolution at 3 μm are needed to establish the extent of blending within the broad feature: it is feasible that structure in the profile could lead to positive identification of several grain constituents. Improved spatial resolution is also desirable, to study positional variations in the feature. Extension of the spectral coverage to other wavelengths will allow investigation of the ice features expected at 6, 12 and 45 μm , and the effect of mantles on the 9.7- μm silicate absorption.

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Stratospheric NO_2 and upper limits of CH_3Cl and C_2H_6 from measurements at 3.4 μm

D. J. W. Kendall

Herzberg Institute of Astrophysics, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6

H. L. Buijs

Bomem Inc., 910 Place Dufour, Vanier, Québec, Canada G1M 3B1

Measurements of stratospheric minor constituent gas concentrations are important for the elucidation of the complex chemistry and the evaluation of the predictions presented by theoretical models regarding the short and long term future of this important atmospheric region. We report here high resolution absorption spectra that have been obtained from a balloon-borne platform in the 3.4- μm spectral region, a region which contains bands due to several atmospheric constituent gas species including methane (CH_4), hydrochloric acid (HCl), nitrogen dioxide (NO_2), methyl chloride (CH_3Cl) and ethane (C_2H_6). From analysis of the atmospheric spectra obtained during sunset, mixing ratio versus altitude profiles have been obtained for HCl (which will be reported elsewhere) and NO_2 , and upper limits have been obtained for the lower stratospheric mixing ratios of CH_3Cl and C_2H_6 .

The balloon flight took place on 27 October 1978, from Alamogordo, New Mexico (33°N) and the present data were obtained during sunset when the balloon was at a float altitude of 39 km. The spectra were obtained with a high resolution (unapodized resolution 0.02 cm^{-1}) Michelson interferometer (Bomem Inc.) featuring an electro-optical servo-controlled dynamical-alignment mechanism. Each full resolution scan was obtained in 48 s and a narrow bandpass filter was utilized in front of the ambient temperature InAs detector to isolate the spectral bandpass in the channel of interest to that shown in Fig. 1. As well as the *P* and *R* branch lines of HCl (marked *P*(1), *R*(0), ..., *R*(5)), the $\nu_1 + \nu_3$ band of NO_2 (refs 1, 2), the ν_1 band of CH_3Cl (ref. 3), and the ν_7 band of C_2H_6 (ref. 4) were covered by the filtering selected. Most of the other features

are due to the ν_3 band of CH_4 (ref. 5) although a few weak non-telluric solar features are evident on detailed analysis. During the flight, the signal strength was considerably weaker than had been anticipated due to a suspected vignetting problem and the resultant spectra exhibited poorer signal-to-noise ratios than had been expected. The three minor constituent gases of interest here exhibit several bands in the IR, however a comparison of their spectral signatures with other more prominent atmospheric absorbers (principally H_2O , CO_2 and O_3) shows that the 3.4- μm region is an excellent region for monitoring of these species by high-resolution atmospheric spectroscopy⁶⁻⁸.

Although the $\nu_1 + \nu_3$ band has been used to determine the abundance of NO_2 in the stratosphere from aircraft observations⁹ and ground-based measurements¹⁰, most concentration profiles have relied on observations of the ν_3 band at 6.2 μm (refs 11-14), or visible band features at 0.45 μm (refs 15-18). Use of the $\nu_1 + \nu_3$ band for solar absorption measurements is attractive due to the fact that the band lies in a region of little absorption by other major atmospheric constituents; however, the band is relatively weak and high spectroscopic resolution is required to identify individual features¹⁰. Furthermore, only recently have laboratory data of the positions and strengths of features within this complex band become available^{1,2,19}. In the present work, 18 individual, well-resolved features between 2,890 and 2,925 cm^{-1} were utilized in conjunction with the line strength values of Toth *et al.*¹⁹ corrected for temperature by multiplying by (296/230). Column density values were obtained for tangent heights between 35 and 19 km and the resultant mixing ratio profile using a 1-km layer-by-layer calculation²⁰ is shown in Fig. 2. Also shown on Fig. 2 are other profiles obtained during sunset from balloon-borne observations (see refs 14-17). Only sunset results have been plotted because the stratospheric concentration profile of NO_2 has been shown to exhibit a large diurnal variation²¹. Note that the profile of Evans *et al.*¹⁷ (see also ref. 18) is the average of the results from four flights obtained during August 1975 and August 1976. As may be seen from Fig. 2, the present result shows a greater concentration of NO_2 below 30 km than other results at the same latitude although the present profile compares well with the higher latitude profiles. This is contrary to theoretical predictions²² and extensive ground-based²³ and aircraft²⁴ observations which indicate a distinct latitudinal dependence of the NO_2 abundance due to a large lower stratospheric concentration

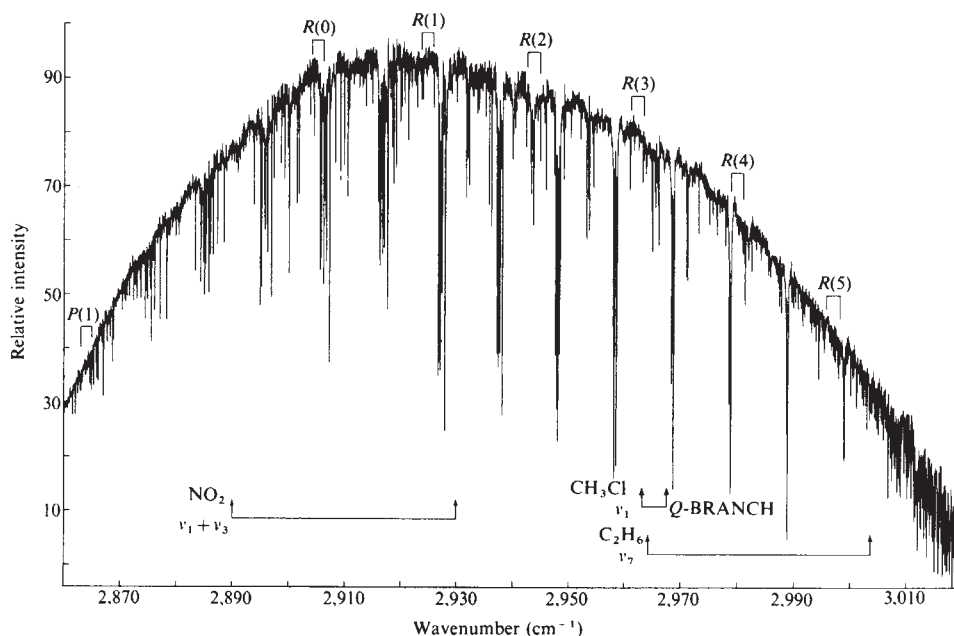


Fig. 1 Spectral bandwidth defined for the HCl channel. Solar depression angle 2.9° from the balloon. HCl 1-0 band line positions are marked as P(1), R(0), ..., R(5).

gradient with respect to latitude, although this dependence appears to be less prominent during the summer²³ and the total column abundance exhibits a dramatic variation from summer to winter at latitudes above 25°N (ref. 24).

Recent laboratory experimental work on the ν_1 band of CH_3Cl has provided accurate positions and strengths of individual Q-branch manifolds^{25,26} which may be used to determine concentrations of this constituent from IR atmospheric measurements. These manifolds are excellent candidates for detection by IR spectrophotometry, as they are relatively isolated and lie in a spectral region containing only weak lines of methane^{6,7}. From analysis of the present spectra, no unambiguous identification of the Q-branch manifolds may be made; however, by determining a minimum detectable equivalent width of $1 \times 10^{-3} \text{ cm}^{-1}$, an upper limit of the volume mixing ratio of 1.25×10^{-9} at the bottom of the stratosphere may be concluded. This value is approximately twice the value determined by recent *in situ* cryosampling experiments with ground-based gas chromatograph-mass spectrometer (GC-MS) analysis^{27,28}.

The spectrum of C_2H_6 in the $3.4\text{-}\mu\text{m}$ spectral region is complex⁴; however, using recent laboratory spectra at a similar

resolution as in the present work⁸, it was hoped that some identifications could be made. However, as in the case of CH_3Cl , no unambiguous identification could be determined and an upper limit of 1×10^{-9} has been placed on the volume mixing ratio of C_2H_6 in the lower stratosphere. This may be compared with a value of similar magnitude obtained recently by a balloon-borne cryosampler²⁹.

Although no positive identifications of CH_3Cl and C_2H_6 have been made in the present work, we feel that with an order of magnitude improvement in sensitivity, which is well within the capabilities of the present instrument, volume mixing ratio profiles of CH_3Cl and C_2H_6 in the stratosphere may be routinely obtained by a remote sensing technique in this spectral region in order to complement the only measurements to date which have been obtained by *in situ* sampling techniques.

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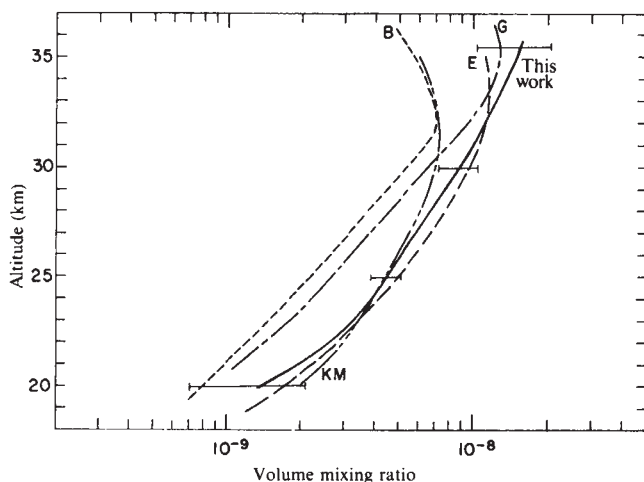


Fig. 2 Volume mixing ratio plotted against altitude profile of NO_2 obtained during sunset from balloon-borne platforms. KM, Kerr and McElroy¹⁵ 56°N July 1974; E, Evans *et al.*¹⁷ 51°N August 1975–August 1976; G, Goldman *et al.*¹⁶ 33°N February 1977; B, Blatherwick *et al.*¹⁴ 33°N October 1979; this work 33°N October 1978.

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Nuclear magnetic resonance of silica polymorphs

Joseph V. Smith

Department of the Geophysical Sciences, The University of Chicago, Chicago, Illinois 60637, USA

C. Scott Blackwell

Union Carbide Corporation, Tarrytown, New York 10591, USA

The chemical bonding in silica polymorphs and silicates is being studied by both experimental and theoretical methods¹. Spinning at the octahedral (magic) angle yields sharp peaks in the NMR patterns for ²⁹Si (ref. 2) and ²⁷Al (ref. 3). We report magic angle spinning (MAS) spectra for seven polymorphs of silica and interpret the chemical shifts empirically in terms of interatomic distances and angles determined by X-ray diffraction. The conclusions provide a partial basis for quantitative interpretation of spectra for framework aluminosilicates, especially zeolite molecular sieves; additional effects from aluminium substitution and non-framework atoms⁴ will need to be evaluated.

Table 1 lists the chemical shifts, interatomic distances and angles for the samples studied. Figure 1 shows the spectra. All samples gave sharp peaks in X-ray powder diffraction patterns, and no impurities were detected. The coesite and stishovite were obtained from the Coconino sandstone shocked by meteorite impact, and are aliquots of samples used for thermochemical studies⁵. The cristobalite is a Geophysical Laboratory standard prepared by heating natural quartz from Lisbon, Maryland, at 1,803 K for 17 h. Hexagonal bipyramids of natural high quartz (F3-21)⁶ were converted into tridymite by heating at 1,673 K for 24 h with Na₂WO₄ flux. A rose quartz from the University of Chicago collection (no. 1: 0.06 wt% Al₂O₃, electron microprobe) did not yield a detectable ²⁷Al peak, but a pale green quartz (49–148, Bernfjord, Iceland⁶) gave a clear signal at 51.0 p.p.m. with spinning sidebands and a hump due to probe background. Such an ²⁷Al chemical shift is consistent with tetrahedral Al. The Bernfjord specimen was selected because the low inversion temperature of 809 K indicated a high Al content^{7,8} which was confirmed by electron microprobe analysis (variable: 0.14–0.6, mean 0.4% Al₂O₃). Fluoride-silicalite (calcined) and its uncalcined tetrapropylammonium fluoride containing precursor^{9,10} (Union Carbide collection) were examined. The complex ²⁹Si NMR spectrum of the calcined material is similar to that reported for silicalite in ref. 3.

No ²⁷Al peak was detected using the same experimental conditions as for quartz. A synthetic silica phase (unpublished results) colloquially known as 'holdstite' because of its retentive nature, gave the same type of X-ray powder pattern as the ZSM-39 phase¹¹. A single-crystal study (J. J. Pluth and J.V.S., in preparation) of the uncalcined holdstite has confirmed it to have the same postulated framework topology and yielded the averaged pseudocubic structure; however, the details of the true structure (trigonal or lower symmetry) are not yet deciphered.

The NMR spectra were obtained on a Bruker CXP-200 solid-state, high-resolution spectrometer. A standard ¹³C constant parity MAS accessory was tuned to 39.7 MHz for ²⁹Si NMR. The ²⁹Si chemical shift is referred to tetramethylsilane. Recalibration for magnet drift was needed only daily, and the shifts should be accurate to at least 0.1 p.p.m. The Andrews-Beam type of spinner was constructed from Delrin and spun with dry nitrogen. The sample spinning rate 3,500 Hz (88 p.p.m. at 39.7 MHz ²⁹Si) obviated spinning sidebands. The magic angle was adjusted by maximizing the spinning sidebands for the ⁷⁹Br resonance in KBr. A typical spectrum was acquired using Block delays from 4 μ s excitation pulses separated by 10.2 s. These conditions ensure sufficient relaxation for quantitative intensities. Cross-polarization was not used. The ²⁷Al spectrum of quartz was obtained with the following conditions: 3- μ s pulses, 1 s delay between pulses and 74,483 scans were co-added. The chemical shift was measured with respect to external [Al(H₂O)₆]³⁺ in Al(NO₃)₃ solution: the spectrum is shown in Fig. 1.

Based on the experimental and theoretical studies of the chemical bonding in silica polymorphs reviewed by Gibbs¹ and Baur¹², empirical correlations were tested between the observed chemical shift and several geometrical parameters. The large negative shift for stishovite (–191 p.p.m.) is explained qualitatively by the octahedral oxygen coordination around each silicon which is associated with a long Si–O bond (0.177 nm)¹³ and strong shielding between adjacent Si atoms. For the silica polymorphs with tetrahedral oxygen coordination around silicon, correlations were sought between the chemical shift and (1) Si–O_j, (2) Si–Si_j, (3) secant (Si–O–Si_j) and (4) [(O_j–Si–O_k)/109.47°], where *j* refers to the four nearest atoms. The angular deformation from ideal tetrahedral geometry proved unpromising and (4) was abandoned. Parameters (2) and (3) are strongly correlated, but are distinct for variable Si–O. Figure 2 shows a weak correlation for (1), a good correlation for (2), and possibly an excellent correlation for (3); however, some uncertainties must be detailed.

Matching of an NMR peak with an Si atom in a crystal structure determination is ambiguous for structures with more than one tetrahedron unless peak heights and population parameters can be related uniquely. The three tetrahedra in holdstite can be matched uniquely from the population ratios (1:4:12), and the measured Si–Si distances are sufficiently accurate even

Table 1 ²⁹Si chemical shifts and atomic parameters

Specimen	Chemical shift (p.p.m.)	Mean Si–O (nm)	Mean Si–Si (nm)	Mean Si–O–Si (deg)
Coesite	–108.1, –113.9	0.16014(3), 0.16124(4) ²⁸	0.3095, 0.3061	152.8, 144.2
Cristobalite	–108.5 (cf –109.9) ²	0.1609(2) ¹⁶	0.3081(1)	146.4(1)
Quartz	–107.1 (cf –107.4) ²	0.1609(1) ²⁹	0.3057(1)	143.6(1)
Stishovite	–191.1	octahedral ¹³ 0.1774	—	—
Tridymite	–109.3 to –114.0, Mean –111.0	0.1584–0.1606, Mean 0.1579 ¹²	0.3063–0.3100, Mean 0.3079	146.7–157.2, Mean 150.0
Holdstite	–108.9, –115.0, –119.4	Not known	0.3074, 0.3091, 0.3125	Not known
Fluoride-silicalite	–108 to –117, Mean –113.2	0.1573–0.1613, Mean 0.1595 ^{9,10}	0.3058–0.3127, Mean 0.3096	146.8–164.1, Mean 154.6
Precursor (uncalcined)	—	—	—	—
Fluoride-silicalite (calcined)	–109.4 to –116.4, Mean –113.4 (cf –109.2 to –116.3 for silicalite) ³	Not known accurately	—	—
(De-aluminated Y)	(–106.9 (ref. 25), –107.4 (ref. 26), –107.8 (ref. 27))	Not known accurately	—	—
(Silica gel)	(–109.3 (ref. 21))	Not considered here	—	—

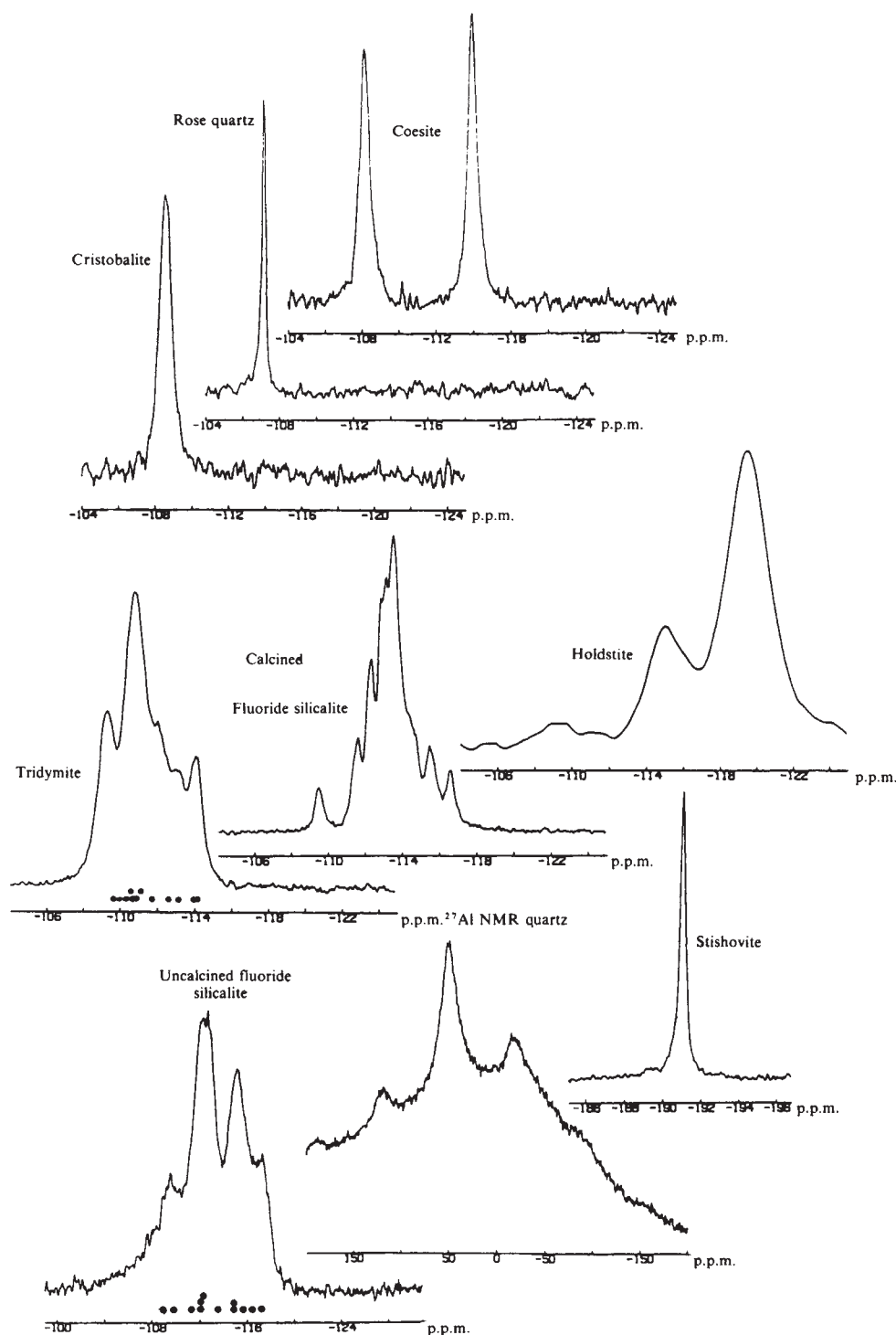


Fig. 1 NMR spectra for silica polymorphs. All are for ^{29}Si except for labelled spectrum for ^{27}Al in quartz. Dots for tridymite and uncalcined fluoride-silicalite show predicted positions using equation (1) and Si-O-Si angles in refs 9, 10, 12.

for the incomplete pseudostructure to warrant addition of the crosses to Fig. 2a. (Note: the distances in ref. 11 for the structurally related ZSM-39 are from a computer simulation.) The two tetrahedra in coesite have equal populations but the ambiguity can be resolved by comparison with the holdsite data and by other considerations. The 12-fold ambiguities from tridymite and calcined fluoride-silicalite cannot be resolved, and the mean value was plotted for each, using graphical averaging of the NMR pattern to obtain the mean chemical shift. Because accurate atomic coordinates are not known for the monoclinic structure of calcined fluoride-silicalite, the coordinates for the uncalcined precursor were used; apparently, the occluded tetrapropylammonium fluoride has only a trivial effect on the mean chemical shift (uncalcined, 113.2; calcined, 113.4). The occluded species in holdsite is assumed also to have no significant effect on the ^{29}Si NMR chemical shift.

Cristobalite poses a problem because of the large difference between the shift (-109.9) listed by Lippmaa *et al.*² and the shift (-108.5) found here. The X-ray powder pattern of the present specimen fits the standard pattern calculated¹⁴ from the crystal structure determination by Dollase¹⁵, which was essentially confirmed by Peacor¹⁶. However, the high-low inversion temperature varies by at least 60 K for different varieties of cristobalite^{17,18}, and various chemical substituents are probably responsible. The present synthetic specimen is essentially pure SiO_2 , and the crystal structure determinations were made on relatively impure natural cristobalites whose structural details are probably different. The cristobalite of Lippmaa *et al.*² should be checked for its chemical composition and for possible polytypic disorder in the tridymite stacking sequence.

In Fig. 2b, the data indicate increased shielding (lower chemical shift; that is, more negative) as the mean Si-O distance

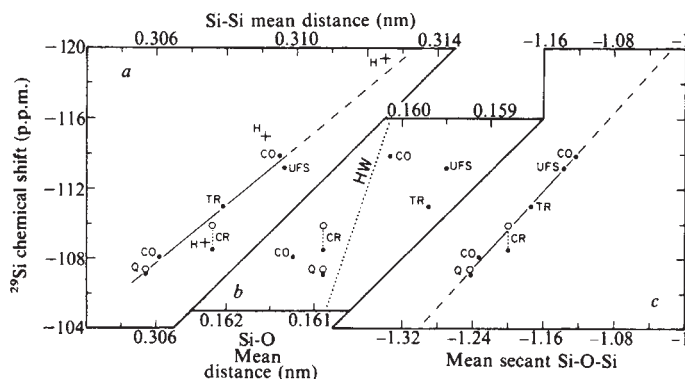


Fig. 2 Relation between chemical shift for ^{29}Si and structural parameters. The continuous lines represent equations (1) and (3), and the dotted line is the equation given in ref. 4: $\text{Si-O (Å)} = 7.29 \times 10^{-4} \delta + 1.6852$. CO, coesite; CR, cristobalite; H, holdsite; Q, quartz; TR, tridymite; UFS, uncalcined fluoride-silicalite. Solid symbols, this work; open symbols, ref. 2.

decreases. There is a scatter of ~ 0.001 nm or 4 p.p.m.; in particular, the data do not match the general equation (dotted line) proposed by Higgins and Woessner⁴ for a range of framework materials. The correlation between chemical shift and mean Si-Si (Fig. 2a) is fairly good when account is taken of the inaccurate crystal structure data for holdsite and the problem for cristobalite, and is even better between chemical shift and mean secant Si-O-Si (Fig. 2c). Indeed, the data for quartz, coesite, mean tridymite and mean uncalcined fluoride-silicalite precursor fit a line within experimental error, as does the datum of Lippmaa *et al.* for cristobalite. Only the present datum for cristobalite is aberrant, and a crystal structure determination for this particular specimen is required. Perhaps the broadness of the peak for the present cristobalite (Fig. 1) indicates an incipient inversion to lower than tetragonal symmetry, with concomitant decrease of the Si-O-Si angle.

Least-squares fits, omitting cristobalite, between the chemical shifts, δ , in p.p.m. (as negative number) and either the mean secant (s) or mean Si-Si distance (S in nanometres) are: $\delta = -176.65 - 55.821s$; correlation 0.99811; standard error 0.21 (1). $s = -3.1571 - 0.017847\delta$; correlation 0.99811; standard error 0.0038 (2). $\delta = 392.10 - 1633.6S$; correlation 0.99382; standard error 0.39 (3). $S = 0.24086 - 0.0006046\delta$; correlation 0.99381; standard error 0.00024 (4).

From the first equation, values of δ were calculated using the mean secants for the 12 tetrahedra in tridymite¹² and uncalcined fluoride-silicalite precursor¹⁰, and plotted as dots below the spectra in Fig. 1. Although the fit is not complete, it is encouraging when account is taken of the unknown effect of minor substituents on the crystal structure of low tridymite, and of the tetrapropylammonium fluoride species inside uncalcined fluoride-silicalite precursor.

The present empirical equations should be tested on other silica polymorphs when appropriate samples become available and accurate crystal structures are determined. Meanwhile, the predicted mean secant (-1.24 ± 0.01) for the range of chemical shifts (-106.9 to -107.8 p.p.m.) for hydrated dealuminated zeolite Y is fairly close to the values of -1.277 and -1.231 for natural hydrated faujasite¹⁹ and dehydrated partly dealuminated zeolite Y at 673 K (ref. 20). The effect of adsorbed water on the NMR spectrum is not known. Taken strictly at face value, the chemical shift of -109.3 for silica gel²¹ would correspond to an Si-O-Si angle of 146° .

We conclude that the present observed range of 12 p.p.m. in the chemical shifts for the various ^{29}Si peaks of the silica polymorphs provides another indication that the interpretation of NMR patterns for framework silicates, and zeolites in particular, must take into account geometrical properties of the framework species as well as the chemical type (see refs 22-24). Further data on the substitution of Al and other minor substituents in silica polymorphs will be given elsewhere.

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Trace metal fronts in European shelf waters

K. Kremling

Institut für Meereskunde, Düsternbrooker Weg 20,
D-2300 Kiel, FRG

The Hebrides shelf edge area is characterized by strong horizontal salinity gradients (fronts) which mark the boundary between Scottish coastal and oceanic waters^{1,2}. The results presented here, obtained in summer 1981 on a transect between the open North Atlantic and the German Bight (Fig. 1), confirm that the hydrographical front is accompanied by dramatic increases in inorganic nutrients (phosphate, silicate) and dissolved trace elements such as Cd, Cu, Mn, and ^{226}Ra (Figs 2 and 3). These data (together with measurements from North Sea regions) suggest that the trace metals are mobilized from partly reduced (organic-rich) sediments and vertically mixed into the surface waters³. The regional variations evident from the transect are interpreted as being the result of the hydrography prevailing in waters around the British Isles⁴.

The near-surface samples (48) were collected aboard the RV *Meteor* on a transect between 28 August and 2 September 1981 (Fig. 1). The water was pumped from 6 m depth (at a rate of $0.3 \text{ m}^3 \text{ h}^{-1}$) through polyethylene tubing (from a towed stainless steel shell suspended underneath the hull) into acid-cleaned

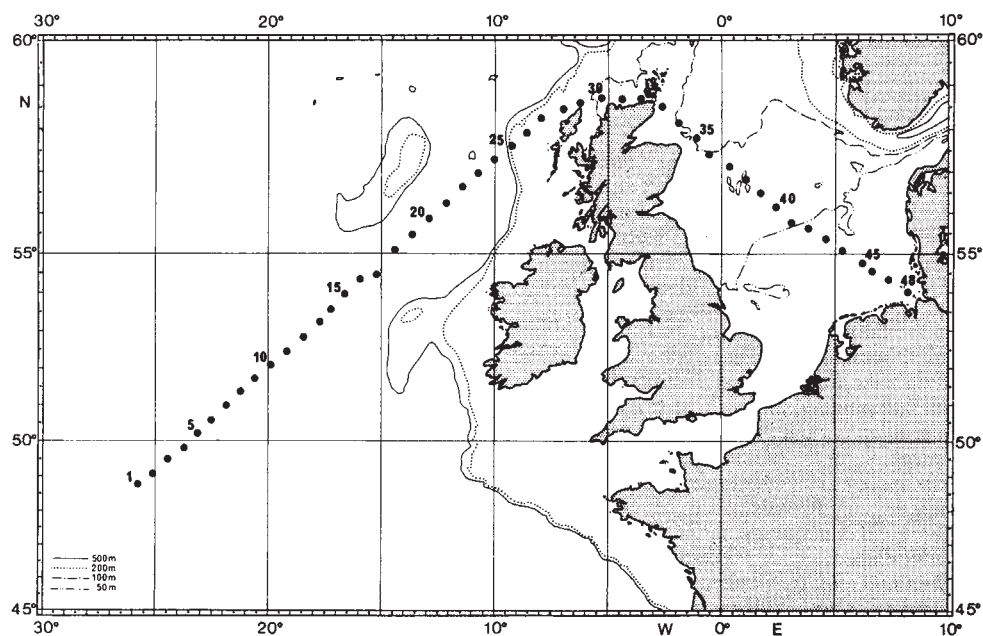


Fig. 1 Surface water sample locations from this study (Stations 1–48).

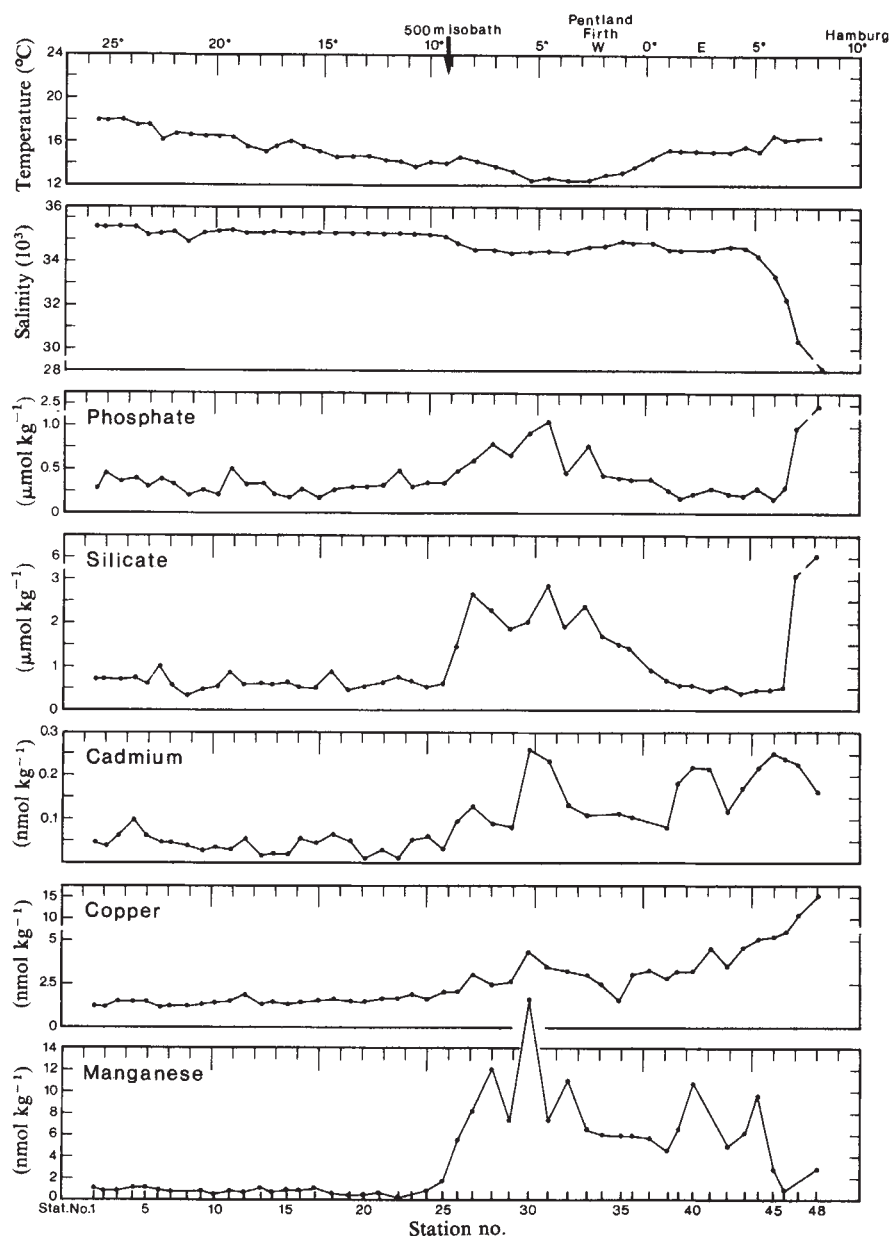


Fig. 2 Surface water data from Stations 1–48 (see Fig. 1).

500-cm³ quartz bottles kept on clean air benches. For storage, the trace-metal samples were immediately acidified with 1 cm³ of quartz-distilled HNO₃ to bring the pH to ~1.7. Filtration of samples (before acidification by means of pressure filtration through cleaned 0.4 µm Nuclepore filters) was started at Station 22 (Fig. 1) since reported particulate concentrations of open North Atlantic waters are only a few per cent of the total dissolved Cd, Cu, and Mn values reported there⁵. The metal analyses were made by flameless atomic absorption spectrometry using the pre-concentration methods described elsewhere^{6,7}. Detection limits (3σ of the blanks) were found at 0.002 (Cd), 0.05 (Cu), and 0.05 (Mn) nmol kg⁻¹; the precision (1σ) was between 10 and 15% at concentrations found in the open Atlantic. The nutrient samples (phosphate, silicate) were immediately analysed following photometric procedures⁸. Salinity was measured using an inductive salinometer.

The results are presented in Fig. 2. Because of the many problems associated with the accurate analysis of certain trace elements, available data for the open North Atlantic and North Sea waters are scarce and patchy. The comparison of the plotted results with the best available data show, however, good agreement for both regions^{2,9-11}. Only the open Atlantic Cd values (Stations 1-24) are higher than those obtained by Bruland and Franks, who in contrast to the present study collected the samples in phosphate depleted surface waters and in a quite different region (Sargasso Sea). The correlation of these elements (nmol Cd = -0.008 + 0.21 [µmol PO₄]; $r = 0.73$ for Stations 1-38 in Fig. 2) is also slightly different from that previously reported for its vertical distribution in Pacific waters (nmol Cd = -0.07 + 0.35 [µmol PO₄])¹².

The striking feature in Fig. 2 is the sharp increase in the nutrient and metal concentrations between Atlantic and shelf waters (marked by a salinity gradient of $\sim 0.6 \times 10^{-3}$ between Stations 25 and 27). The frontal structure of the chemical properties is also apparent from the simultaneous increase of ²²⁶Ra (Fig. 3)¹³, a radionuclide showing generally much higher values in bottom than in surface waters^{14,15}. The appearance of these fronts (and the metal peaks in the North Sea) may be explained by the combined physical and geochemical processes operating over the shelf area. It is well known^{4,16} that the tidal currents west of Britain and in the North Sea enhance mixing to a degree that some regions are vertically homogeneous throughout the year whereas others are seasonally stratified, thus preventing continuous transfer of properties from near bottom waters into the upper layers during the summer. Therefore, local and seasonal variations in the surface values over the shelf area should be interpreted taking into account combined causes within the surface and the bottom water.

This interpretation is supported by results of this transect which passed through an area of intense tidal mixing (the Northern Scotland-Shetland area) into regions with the normal summer stratification (interrupted, however, by 'islands' of totally mixed water bodies¹⁶) before entering the German Bight. The high phosphate and silicate concentrations in the mixed waters (Fig. 2) could be attributed to biogeochemical regeneration (with enrichment in deeper waters). The Cd, Cu, and Mn peaks over the shelf, however, cannot be explained by this mechanism. Vertical profiles in the Atlantic Ocean¹⁰ show that, for example, values as high as 4.2 or 18.4 (nmol kg⁻¹) for Cu and Mn (Station 30) are never reached in the deep waters. The most plausible explanation is remobilization from the shelf sediments. This happens, when oxygen in sediments, which are rich in organic material (mainly originating from riverborne detritus and quite significant in the shelf area²) is depleted by biological processes, and the manganese and iron oxides are reduced to Mn²⁺ and Fe²⁺. These ions migrate into the pore waters of the sediment and then diffuse (or become resuspended by turbulent mixing) into the waters overlying the bottom^{7,17-20}. The mechanisms for the Cd and Cu release, however, are not necessarily associated with the reduction of the manganese and iron oxides. Although very little is known about the geochemistry of Cd in partly reduced sediments, there is strong

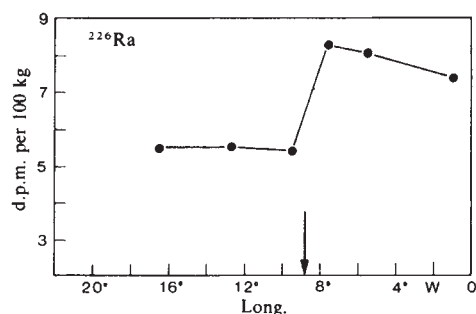


Fig. 3 Surface ²²⁶Ra data collected on the transect by Kromer and Roether¹³ (with indicated 200-m isobath).

evidence supporting the suggestion that Cu is probably released during the early diagenesis of organic matter at the sediment surface^{10,21,22}.

Alternative sources that could, for example, produce the trace metal enrichments in the Hebrides shelf waters (Stations 26-30) can be ruled out. (1) An aeolian input could hardly produce these concentration levels with such distinct boundaries. (2) Rivers with a significant discharge of metals do not exist in this area (the low salinity in the coastal waters results from the excess of precipitation compared with evaporation). (3) Drainage from terrestrial sources (sewage or industrial waste) can be dismissed on two grounds: first, the dominance of manganese relative to cadmium and copper (Fig. 2) is very atypical for such spills; second, the elements should be uniformly mixed after migration into the shelf area (similar to the salinity which is horizontally homogeneous between Stations 27-32 in Fig. 2).

I conclude that the remobilization of trace metals from partly reduced sediments and subsequent mixing into the surface waters is a subject for major concern in waters around the British Isles. These processes are probably variable, depending on seasonal changes in the currents and hydrography and perhaps even on temporal variations in the chemistry and biology of the shelf sediments. The variations in the metal versus location plots (Fig. 2) may be interpreted in this way, but would have to be studied further to support this hypothesis.

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Large particle transport of plutonium and other fallout radionuclides to the deep ocean

H. D. Livingston & R. F. Anderson*

Department of Chemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

Artificial radionuclides introduced to the atmosphere as a consequence of atmospheric nuclear weapons tests have been detected in deep sediments of the Atlantic¹ and Pacific² Oceans and of the Mediterranean Sea³. The nuclides which have been found in these sediments have half lives long enough to be detectable 10 or more years after their introduction to the ocean and biogeochemistries which permit particle association and transport. They include such nuclides as ^{239,240}Pu, ²³⁸Pu, ²⁴¹Am, ⁵⁵Fe and ¹³⁷Cs (refs 1–5). For fallout nuclides to have been transported to the deep ocean in the two to three decades since they were delivered to the sea surface, relatively rapid transport through the water column is required. We describe here the results of radiochemical analyses of Pu and other fallout nuclides in three series of sediment trap samples collected as part of the PARFLUX program^{6–8}. The data are used to consider the role that large particles have in processes which transfer particle reactive artificial radionuclides through oceanic water columns.

¹³⁷Cs, although readily transported to, and measurable in, deep ocean sediments, behaves in oceanic water columns predominantly as a 'soluble' nuclide and has properties which make it useful as a water tracer comparable to ⁹⁰Sr and ³H (ref. 9). Measurements of the vertical distribution of fallout plutonium in both the Atlantic^{4,10} and Pacific¹¹ Oceans have shown that plutonium has been moved vertically downwards relative to the distributions of such soluble tracers as ⁹⁰Sr or ¹³⁷Cs. In the Pacific this process has produced a widespread, shallow subsurface Pu-rich layer with a concentration maximum at ~465 m in 1974¹¹ and another Pu-rich layer in the water mass extending to about 1,000 m above abyssal sediments¹¹. These features of the vertical water column distribution of Pu and the presence of Pu in deep ocean sediments are a direct result of its transport on sinking particles. However, most of the Pu in open-ocean water columns is present in an unfilterable form with essentially soluble characteristics¹².

The sediment traps were deployed (Table 1) at two open ocean sites in equatorial regions of both the North Atlantic (PARFLUX E) and North Pacific (PARFLUX P) Oceans, and in the Panama Basin (PARFLUX PB), a biologically productive ocean margin region. Collection details are summarized in Table 1 and are discussed more fully elsewhere^{6,7} as is the relevant hydrography (P. G. Brewer *et al.*, and J. K. B. Bishop and D. W. Spencer, in preparation). The material analysed from the sediment traps was in a size fraction <1 mm. Particles >1 mm in diameter, which were removed from the samples for separate studies, constituted a significant portion of the total sample only for the shallowest trap at each site⁶. Radiochemical analyses were undertaken on dried subsamples of refrigerated collected material. The radiochemical methods used in the analyses of the dried sediment trap material¹³ are essentially the same as those described elsewhere for marine sediment analyses^{14–16}.

Most of the particulate material collected by the sediment traps at all depths consisted of large, rapidly settling aggregates of predominantly biogenic material formed at, or near, the sea surface^{6,8}. The composition and particle size-frequency distribution

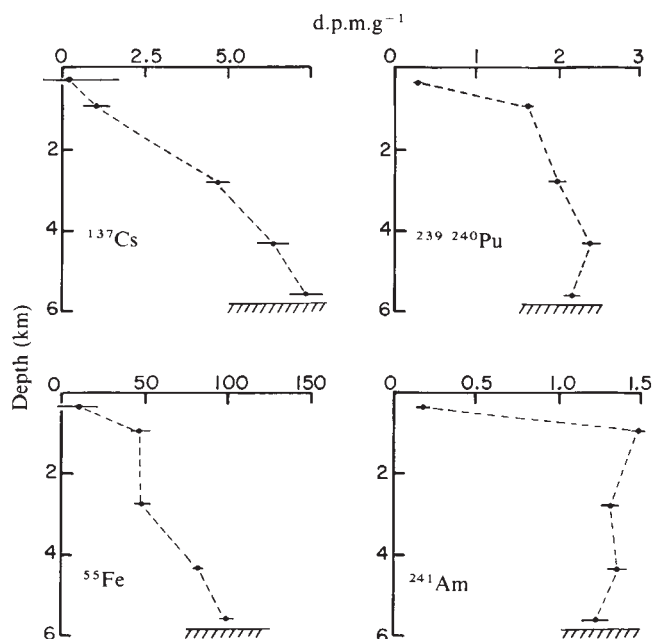


Fig. 1 Nuclide concentrations (d.p.m.g⁻¹) in PARFLUX P sediment trap samples.

are described elsewhere^{6–8}. The high organic content of the trapped material and the fresh nature of its skeletal calcium carbonate components provide evidence of its rapid transport after formation at the sea surface^{6,8}. The similarity of the specific activities and fluxes of particulate Pu throughout the water column at each site (Table 1) suggests that the particles acquire their plutonium in the upper kilometre where the water concentrations are high¹¹, and serve as vehicles for fast transport to the deep ocean. The low specific activity and flux of Pu in the Site P 378-m sample may reflect the position of the sediment trap slightly above the Pu concentration maximum in the Pacific¹¹.

Plutonium inventories in the water column are much greater in the tropical Pacific¹¹ than in the Atlantic (V. T. Bowen, unpublished results) because substantial amounts of Pu were delivered to the Pacific from close-in fallout originating at the US Marshall Islands test sites¹¹. Fluxes of particulate ^{239,240}Pu are several-fold greater at Site P than at Site E (Table 1), reflecting the differing water column inventories of 2.1 (ref. 11) and 0.48 (V. T. Bowen, unpublished results) mCi km⁻² respectively of total (dissolved plus particulate) ^{239,240}Pu. Using average deep sea ^{239,240}Pu fluxes of 2.5 and 10.0 d.p.m. m⁻² yr⁻¹ at Sites E and P respectively (Table 1), we obtain apparent Pu residence times (inventory/flux) of 417 yr and 462 yr. Residence times of a few hundred years are consistent with the observation that the Pu distribution in the North Pacific did not change measurably over a period of several years¹¹.

Particulate ^{239,240}Pu fluxes are much greater at Site PB than at either open ocean site (Table 1). Water column data are not available for Site PB, but the Pu inventory should be less than at Site P based on the pattern of Pu inventories measured over a wide area of the North Pacific¹¹. We believe that the high Pu fluxes reflect a net lateral transport of dissolved Pu from the open ocean to this ocean margin site. Inventories of plutonium in sediments from the west coasts of North^{17,18} and South¹⁹ America are greater than would be expected if supply were exclusively by atmospheric fallout. ²¹⁰Pb inventories in sediments collected off the north-west US coast are also several times greater than would be supplied by atmospheric deposition of ²¹⁰Pb alone²⁰. Removal of Pu and ²¹⁰Pb following lateral transport from the open ocean is the only reasonable mechanism that could produce the high inventories of these nuclides in the coastal sediments^{18,20}. Similarly, supply to ocean-margin sediments by lateral transport from the open ocean has also been

* Present address: Lamont-Doherty Geological Observatory, Palisades, New York 10964, USA.

Table 1 Material fluxes, $^{239,240}\text{Pu}$ specific activities and fluxes for sediment trap samples

Trap deployment	Trap depth (m)	$^{239,240}\text{Pu}$ specific activity (d.p.m. g $^{-1}$)	Particle flux (g m $^{-2}$ yr $^{-1}$)	$^{239,240}\text{Pu}$ flux (d.p.m. m $^{-2}$ yr)
Equatorial north-west Atlantic	389	0.20 $\pm$ 0.02	18.9	3.8 $\pm$ 0.4
13°05' N 54° W	988	0.14 $\pm$ 0.02	16.1	2.2 $\pm$ 0.3
16 November 1977–26 February 1978	3,755	0.15 $\pm$ 0.02	19.6	2.9 $\pm$ 0.4
Water depth 5,288 m (PARFLUX E)	5,086	0.12 $\pm$ 0.02	18.1	2.2 $\pm$ 0.4
Equatorial North Pacific	378	0.28 $\pm$ 0.03	1.82	0.50 $\pm$ 0.05
15°21' N 151° W	978	1.61 $\pm$ 0.09	2.68	4.3 $\pm$ 0.2
18 September–18 November 1978	2,778	1.98 $\pm$ 0.10	6.06	12.0 $\pm$ 0.6
Water depth 5,792 m (PARFLUX P)	4,280	2.39 $\pm$ 0.13	5.64	13.5 $\pm$ 0.7
	5,582	2.15 $\pm$ 0.10	3.55	7.6 $\pm$ 0.4
Panama Basin	667	0.42 $\pm$ 0.03	41.8	18 $\pm$ 1
5°6' N 81°32' W	2,265	0.50 $\pm$ 0.03	46.1	23 $\pm$ 2
August–November 1979	2,869	0.42 $\pm$ 0.02	58.3	25 $\pm$ 2
Water depth 3,880 m (PARFLUX PB)	3,769	0.29 $\pm$ 0.02	65.9	19 $\pm$ 1
	3,791	0.32 $\pm$ 0.03	67.1	21 $\pm$ 2

Table 2 Specific activities and fluxes of $^{239,240}\text{Pu}$, ^{241}Am , ^{137}Cs , ^{90}Sr and ^{55}Fe in Site P sediment trap samples

Depth (m)	$^{239,240}\text{Pu}$	^{241}Am	^{137}Cs (d.p.m. g $^{-1}$)	^{90}Sr	$^{55}\text{Fe}^*$	$^{239,240}\text{Pu}$	^{241}Am (d.p.m. m $^{-2}$ yr $^{-1}$)	^{137}Cs	$^{55}\text{Fe}^*$
378	0.28 $\pm$ 0.03	0.18 $\pm$ 0.04	0.2 $\pm$ 1.5	–5 $\pm$ 6	10 $\pm$ 11	0.50 $\pm$ 0.05	0.33 $\pm$ 0.07	0.4 $\pm$ 2.7	18 $\pm$ 20
978	1.61 $\pm$ 0.09	1.49 $\pm$ 0.05	1.4 $\pm$ 0.5	1.3 $\pm$ 0.2	47 $\pm$ 6	4.3 $\pm$ 0.2	4.00 $\pm$ 0.13	2.7 $\pm$ 1.1	126 $\pm$ 16
2,778	1.98 $\pm$ 0.10	1.31 $\pm$ 0.11	4.7 $\pm$ 0.4	ND	48 $\pm$ 3	12.0 $\pm$ 0.6	7.94 $\pm$ 0.67	28 $\pm$ 2	291 $\pm$ 18
4,280	2.39 $\pm$ 0.13	1.37 $\pm$ 0.07	6.3 $\pm$ 0.5	–1.0 $\pm$ 1.2	82 $\pm$ 3	13.5 $\pm$ 0.7	7.73 $\pm$ 0.39	36 $\pm$ 3	462 $\pm$ 17
5,582	2.15 $\pm$ 0.10	1.21 $\pm$ 0.08	7.3 $\pm$ 0.5	<5	98 $\pm$ 6	7.6 $\pm$ 0.4	4.30 $\pm$ 0.28	26 $\pm$ 2	348 $\pm$ 21

ND, not determined.

* Corrected for decay to reference date 1 January 1975.

observed during studies of ^{230}Th and ^{231}Pa in the Panama Basin²¹ and off north-west Africa²². Particle fluxes in coastal waters are generally greater than in the open ocean because of higher biological productivity and supply of material from the continents. Metal removal by adsorption to particles is therefore enhanced in coastal waters, permitting horizontal mixing processes to cause a net transport of dissolved particle-reactive elements such as Pu, Pb, Th and Pa from the open ocean to ocean margins, with subsequent deposition of these elements in margin sediments. This is probably a common phenomenon which is also likely to be an important factor in the geochemical cycling of other reactive trace elements as well.

Specific activities and fluxes of all the fallout radionuclides measured in Site P samples are presented in Table 2. ^{90}Sr activities in every case were below detection limits, consistent with the soluble nature of Sr in seawater. In contrast to $^{239,240}\text{Pu}$ and ^{241}Am , whose specific activities vary little below 978 m, specific activities of both ^{137}Cs and ^{55}Fe increase with increasing trap depth (Fig. 1). Possible reasons for this are discussed below.

Comparing nuclide ratios in sediment trap samples with the corresponding ratios in the water column and in nearby surface sediments (Table 3) provides interesting insight into the particle transport and fractionation of these elements. $^{238}\text{Pu}/^{239,240}\text{Pu}$ ratios are typical of Northern Hemisphere fallout influenced by the burn-up of SNAP 9A²³. The $^{241}\text{Am}/^{239,240}\text{Pu}$ ratio is uniform in particulate material collected throughout the water column, except at 978 m, and is close to the ratio measured in surface sediments. The particulate $^{241}\text{Am}/^{239,240}\text{Pu}$ ratio is about twice the dissolved ratio, consistent with the conclusion that Am is more reactive than Pu towards uptake by particles^{4,24,25}. Am/Pu ratios in fine particulate matter collected by filtering large volumes of seawater in the North Pacific increased from <1 at the sea surface to >2 between 500 m and 1,500 m, and then decreased back to <1 at 3,000 m (ref. 26). Apparently enough of this fine particulate matter was incorporated into the 978-m sample to alter its Am/Pu ratio. The chemical processes altering the fine particulate Am/Pu ratio as a function of depth are as yet undetermined.

Particulate $^{239,240}\text{Pu}/^{137}\text{Cs}$ ratios in the sediment trap samples are an order of magnitude or more greater than the ratio in the water column (Table 3), evidence of the much greater solubility of Cs than of Pu with respect to removal by adsorption to particles. The particulate Pu/Cs ratio decreases significantly with depth, reflecting the corresponding increase in ^{137}Cs activity. ^{137}Cs concentrations in North Pacific seawater below 1,000 m are at most a few per cent of surface water values^{11,27}. Therefore, if particulate Pu/Cs ratios decrease and if ^{137}Cs activities increase with depth because particles are removing ^{137}Cs from deep water, then ^{137}Cs exists in a much less soluble form in the deep ocean than in surface seawater. Alternatively, if dissolution of Pu without additional uptake of ^{137}Cs reduces particulate Pu/Cs ratios with depth (Table 3), then increasing particulate ^{137}Cs activities (Table 2) must result from retention of ^{137}Cs by refractory material while labile components are lost from settling particles. In addition, most of the particulate Pu settling below 978 m must redissolve before the particles reach the deepest trap at 5,582 m. However, it is difficult to explain the five-fold increase in particulate ^{137}Cs activity between 978 m and 5,582 m exclusively by the latter mechanism because there is no evidence to support an 80% loss of labile particle flux between these depths⁶. Both processes, acquisition of insoluble ^{137}Cs and dissolution of Pu, might occur simultaneously as particles settle through the water column.

^{137}Cs in the trapped material was not removed from ^{137}Cs -rich surface waters by simple ion exchange processes. If this were the case, then ^{137}Cs would be displaced from particle surfaces by stable Cs in the ^{137}Cs -poor deep waters. Rather, particulate ^{137}Cs in the deep Pacific must be in a nonexchangeable form. One possible scenario is that most of the total ^{137}Cs in the deep Pacific exists in small (of the order of 1 μm) refractory particulate phases which are aggregated by large (tens to hundreds of micrometres) rapidly settling particles^{28,29}. A similar aggregation process has been proposed (D. W. Spencer *et al.*, in preparation) to explain the increase with depth in aluminum content of these sediment trap samples^{7,8}. Preliminary results for two samples of predominantly fine particulate matter filtered from

Table 3 Comparison of nuclide ratios in the water column, in trapped particles, and in surface sediments

Sample	$^{238}\text{Pu}/^{239,240}\text{Pu}$ ($\times 100$)	$^{241}\text{Am}/^{239,240}\text{Pu}$	$^{239,240}\text{Pu}/^{137}\text{Cs}$	$^{55}\text{Fe}/^{239,240}\text{Pu}$
Water column				
GEOSECS 235	1.1–4.2 (ref. 33)	0.3*	0.032 (ref. 11)	ND
P-378 m	5 $\pm$ 4	0.64 $\pm$ 0.16	1 $\pm$ 10	36 $\pm$ 39
P-978	3.3 $\pm$ 0.7	0.93 $\pm$ 0.06	1.2 $\pm$ 0.4	29 $\pm$ 4
P-2,778 m	3.8 $\pm$ 0.5	0.66 $\pm$ 0.06	0.42 $\pm$ 0.04	24 $\pm$ 2
P-4,280 m	3.6 $\pm$ 0.5	0.57 $\pm$ 0.04	0.38 $\pm$ 0.04	34 $\pm$ 2
P-5,582 m	4.9 $\pm$ 0.7	0.56 $\pm$ 0.05	0.29 $\pm$ 0.02	46 $\pm$ 4
Sediments: average of 18 cores at 30° N, 160° W	9*	0.52 (ref. 32)	0.06 (refs 32, 33)	95*

ND, not determined.

* V. T. Bowen, unpublished results.

large volumes of seawater at a North Pacific station (sample depths 5,600 and 5,800m) give average particulate ^{137}Cs and $^{239,240}\text{Pu}$ concentrations of 0.1 and 0.003 d.p.m. per 100 l respectively. Using near-bottom average particulate matter concentrations from Site P of $\sim 10 \mu\text{g l}^{-1}$ (P. Brewer, unpublished results) we obtain Cs and Pu activities of ~ 100 and ~ 3 disintegrations per minute per gram (d.p.m. g^{-1}) respectively. Incorporation of some of these fine particles into large aggregates collected by the sediment traps would explain the increasing Cs activities and decreasing Pu/Cs ratios with depth (Tables 2, 3).

The fivefold drop in the Pu/Cs ratio between the deepest Site P sediment trap sample and North Pacific surface sediments (Table 3) could result either from resolubilization of most of the particulate Pu delivered to the sea floor, or from a large input to the sediments of refractory ^{137}Cs during the early days of weapons testing. The mechanism of preferential resolubilization of particulate Pu at the interface is favoured by the presence of elevated concentrations of Pu, with primarily oxidized and soluble properties, in near-bottom seawater²⁶. If Pu/Cs ratios in our sediment trap samples are representative of particulate Pu/Cs ratios during the past 20 yr, then $\sim 80\%$ of the particulate Pu reaching the sea floor is remobilized. Desorption from particles in the deep ocean has been demonstrated for thorium^{30,31}, a tetravalent actinide with some chemical similarities to plutonium in its least soluble oxidation state (IV). Therefore, it is not surprising that particulate Pu might also redissolve. However, we are left with the unexpected conclusion that ^{137}Cs in the deep ocean is much less soluble than plutonium.

$^{55}\text{Fe}/^{239,240}\text{Pu}$ ratios (Table 3) increase with depth in our sediment trap samples, and increase further by a factor of 2 between the deepest trap sample and surface sediments. We are presented with the same situation as described above for ^{137}Cs , that particles must either acquire additional ^{55}Fe or lose $^{239,240}\text{Pu}$ as they settle through the deep water column. As far as we know the ratio in the water column has not been reported.

Finally, present fluxes of particulate Pu and Cs can be compared with sediment inventories of these nuclides to extract information regarding fluxes in the past. Average Pu and Cs inventories for a suite of 18 cores collected between 1974 and 1979 at 30° N, 160° W are 129 and 2,130 d.p.m. m^{-2} respectively^{32,33}. We assume that the inventories in the sediments are proportional to the inventories in the water column so we can correct for latitudinal differences using GEOSECS data¹¹. Sediment inventories for 15° N (Site P), estimated as the ratio of the water column inventory at GEOSECS Station 235 to the inventory at Station 212 times the measured sediment inventory at 30° N, are 97 and 1260 d.p.m. m^{-2} for Pu and Cs respectively. Twenty years delivery (1958–78) at our measured (1978) fluxes would produce sediment inventories of 256 and 640 d.p.m. m^{-2} for Pu and Cs respectively. Therefore, even if no particulate Cs dissolves at the sediment–water interface, ^{137}Cs fluxes must have been higher in the past than in 1978 to produce this sediment inventory. Present Pu fluxes overestimate sediment inventories, so either Pu fluxes were much lower in the past than at present or most of the particulate Pu flux dissolved at

the sediment–water interface. The latter explanation is more reasonable because particulate Pu fluxes should have been greater in the past when a larger portion of fallout Pu was present in biologically active surface waters, and because inventories of dissolved Pu in the bottom 1 km of seawater are ~ 2 –5 times greater than the sediment average²⁶. This dissolved Pu must have been supplied by a flux of particles from the sea surface.

Remobilization of a large portion of the particulate Pu delivered to the deep ocean is strongly supported by the available evidence. ^{241}Am must be similarly remobilized because particulate $^{241}\text{Am}/^{239,240}\text{Pu}$ ratios are uniform between the deep water and the sediments. The possibility that refractory particulate forms of ^{137}Cs and ^{55}Fe are being removed from the deep ocean by aggregation with large particles requires further investigation.

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Glacial–Holocene transition in deep-sea sediments: manganese-spike in the east-equatorial Pacific

W. H. Berger*, R. C. Finkel*, J. S. Killingley* & V. Marchig†

* Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA

† Federal Institute for Geosciences and Natural Resources, D-3000 Hannover, FRG

Over large areas of the tropical Pacific, calcareous sediments show one or more dark-coloured manganese-rich layers within the uppermost 40 cm of the record^{1,2}. In the east-equatorial Pacific two layers are strongly developed, with the upper one appearing as a double layer in places. We report here that the lower layer is centred on the glacial–Holocene boundary, and we present evidence that its origin is tied to a pronounced drop in fertility of the ocean. Such a fertility decrease is implicit in a weakening of east-equatorial upwelling systems^{3,4}. If real, it has important ramifications for the history of oceanic (and atmospheric) chemistry.

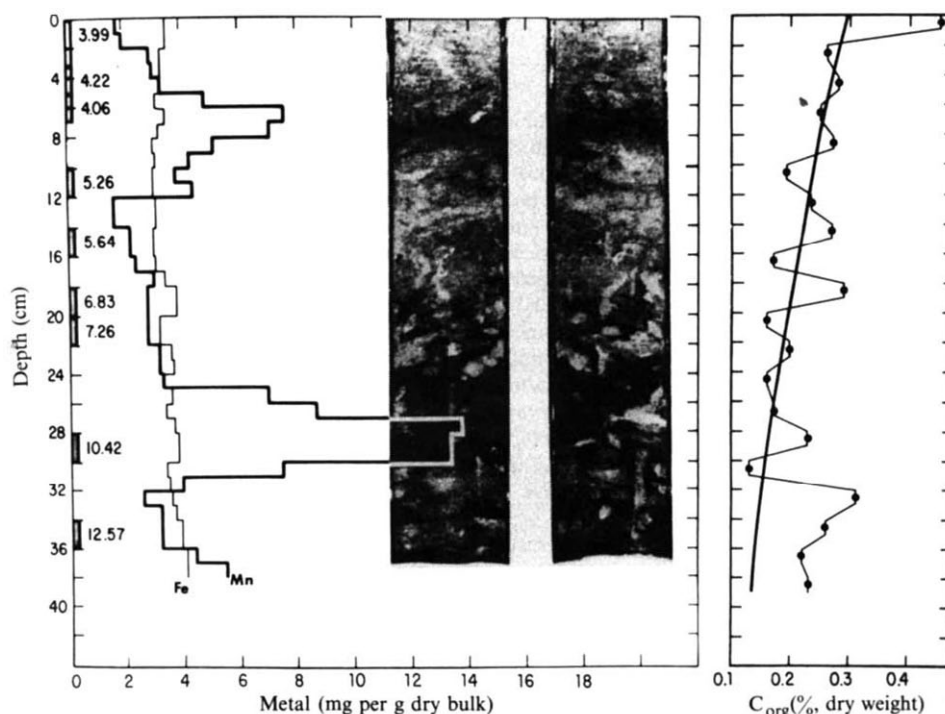
We analysed five box cores (PLDS 66, 68, 70, 72 and 74) all from water depths near 4,000 m close to the Equator, and from between 100° W and 115° W; Core PLDS 72 may be taken as typical. Carbonate percentages, physical properties, and diatom stratigraphy have already been published for this core^{5–7}. The dark layers (Fig. 1) occur at two sites: at 7–10 cm down-core, near the bottom of the benthic mixed layer (subsurface Mn-spike), and near 30 cm down-core, at a level corresponding to the glacial–Holocene boundary (deglacial Mn-spike). Three sources of pigment commonly generate dark layers in pelagic sediments: organic carbon, iron compounds,

and manganese compounds. Of these, only Mn-distributions coincide with the colour-distribution within the core (Fig. 1). Thus, each dark layer constitutes a manganese-spike.

A commonly accepted explanation for subsurface manganese concentrations is that they are the result of Mn migration within the sediment, along redox gradients^{8–12}. Our observations agree with this proposition. The manganese which is responsible for the dark-layer phenomenon is or was mobile. Many burrows (*Planolites*) are light-coloured, even when situated within the Mn-spike horizons (Fig. 1). Burrows may be thought of as conduits for organic matter transport from the sediment surface to below the mixed layer¹³. They provide a microenvironment where Mn can be reduced and hence remobilized^{5,13,14}. Further evidence for the mobility of Mn within the cores comes from the inspection of the foraminiferal assemblage under the microscope. Many foraminiferal shells show minute dark specks on their surface, which resemble micronodules. The surface of calcareous shells, within a given microenvironment, represent sites of elevated pH. It is expected, from the stability-field of Mn²⁺, and the pH-dependency of oxidation rates¹⁵ that manganese should preferentially precipitate on such a surface. We found a particularly high incidence of speckled shells at 27–28 cm depth in the core. As the foraminifera shells are clean when first arriving at the sea floor, these specks are evidence for diagenetic mobility and reprecipitation of manganese within the sediment.

The origin of the subsurface Mn-spike can be derived from Mn remobilization at depth, upward migration, and precipitation at a subsurface horizon where the redox potential (E_h) reaches a critical value. Extant steady-state models are based on molecular diffusion^{16–18}. Our data suggest that Mn remobilization by organic matter is strongly influenced by the action of benthic burrowing organisms. The organic carbon distribution in PLDS 72 (Fig. 1) shows high concentrations in the surface 1 cm of sediment. The ¹⁴C distribution indicates that occasional ‘lumpy mixing’⁵ by large burrowers extends to below 12 cm (see Fig. 1, ¹⁴C distribution). Homogenizing mixing extends to between 6 and 8 cm (ref. 5). The bioturbation rate (of the order of 0.1 cm² yr^{–1}; ref. 19) is much slower than porewater molecular diffusion (30 cm² yr^{–1}; ref. 13), so that bioturbation cannot control porewater redox potentials by direct advection. However, bioturbation produces high porosity, as well as conduits for seawater penetration. Therefore, the agreement between the depth of homogenizing mixing and the depth of the subsurface Mn spike may be more than coincidental. Large

Fig. 1 Radiocarbon dates (3,990–12,570 yr), Mn and Fe concentrations (mg per g), visual appearance, and organic carbon content (%) of box core PLDS 72. (Sub-core shown in photograph is shorter than sub-cores sampled.)



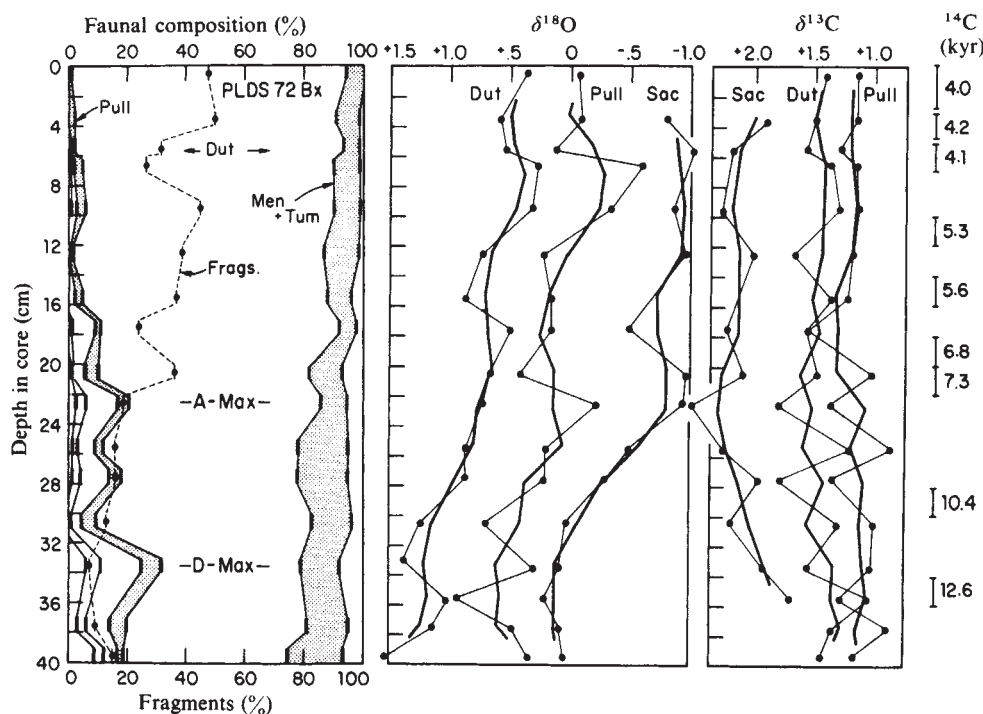


Fig. 2 Faunal composition, oxygen isotopes, carbon isotopes, and radiocarbon stratigraphy of box core PLDS 72 Bx. Faunal composition refers to planktonic foraminifera 250–420 μm . From left to right: *G. ruber*, *G. siphonifera*, *G. sacculifer*, *P. obliquiloculata* (Pull), *N. dutertrei* (Dut), *G. menardii* + *G. tumida* (Men + Tum), other. *N. dutertrei* dominates throughout. Frags., percentage fragments; D-Max, deglacial preservation maximum; A-Max, althothermal preservation maximum. Stable isotopes refer to planktonic foraminifera *N. dutertrei*, *P. obliquiloculata*, and *G. sacculifer*, of size 250–420 μm . The heavy lines are three-point moving averages.

burrowers, by transporting organic-rich surficial sediment below 10 cm depth, together with faecal matter and mucosal burrow lining, can, in effect, pump manganese into the subsurface Mn spike. Manganese together with sufficient reducing agent arrives below the spike horizon, diffuses upward, and feeds the subsurface Mn spike. The efficiency of the pump depends on fertility, which controls depth and intensity of bioturbation. Some Mn in the upper layer may also be derived from deeper in the sediment, through long-distance migration.

An alternative hypothesis that the subsurface Mn layer is produced by increased Mn supply sometime in the past, is unattractive. First, had there been increased supply between 5,000 and 4,000 yr ago, we should see much more Mn in the mixed layer above the Mn spike: this sediment has the same ^{14}C age as the sediment containing the spike. Second, the Mn accumulation rates within the Mn peaks of the Pleiades cores do not show the expected anti-correlation with distance from the East Pacific Rise, (EPR) for hydrothermal injection at the ridge crest. Third, we should expect at least some increase in Fe-accumulation during Mn peak times, since the EPR emits Fe as well as Mn (refs 20–22). There is no evidence for fluctuations in Fe supply.

The origin of the deglacial Mn spike may be derived as follows. The lower dark layer is similar to the upper dark layer in all respects except for being darker and containing a higher percentage of Mn. We see no reason, therefore, to call on a new mechanism to produce this layer, and argue that it is a fossil subsurface spike. We rule out the possibility of a diastem phenomenon because of continuous sedimentation rates ($2.79 \times 10^{-3} \text{ cm yr}^{-1}$ below the layer and $2.53 \times 10^{-3} \text{ cm yr}^{-1}$ above it), and because of high carbonate values in the critical interval ($77\% \pm 3\%$; ref. 7). Furthermore, foraminiferal preservation is at a peak just below the Mn-spike horizon (Fig. 2) indicating a well-behaved stratigraphy (the preservation spike is in the correct position²³). As before, we rule out the argument for a short period of increased Mn supply, by invoking the lack of correlation with distance from the EPR, and the static Fe stratigraphy.

What controls the amplitude of the subsurface Mn spike? If our model of Mn pumping by burrowers is correct, then the rate at which organic matter is transferred from the upper layer of homogeneous mixing to the lower layer of lumpy mixing is crucial. It, and the sedimentation rate, determine the maximum size of the Mn buildup between the two tiers of mixing. A high ratio of the rate of downward transfer to the rate of sediment

accumulation is favourable for the buildup. Also, the length of time the process has been working is important, in cases where the equilibrium value of the buildup has not been reached. We conclude that the benthic Mn pump must have been very efficient in pre-Holocene time, and that there was sufficient time to achieve a high Mn value. Because burrowing intensity is limited by food supply⁶ we deduce that the ocean's productivity was substantially higher during glacial time than during the Holocene, in this region. To explain the fossilization of a portion of the former steady-state subsurface spike, we postulate a sudden drop in fertility during deglaciation. Both the flux of organic carbon to the sea floor and the burrowing activity subsided during the glacial–Holocene transition. The reduction rate within the steady-state Mn layer was then unable to provide for continual reerosion of Mn, which is necessary to keep it migrating upwards.

There are independent indications that fertility of surface waters dropped considerably during deglaciation, from organic carbon stratigraphy²⁴ and from general geochemical arguments involving the rise in atmospheric P_{CO_2} during deglaciation²⁵. The data available for Core PLDS 72 also support our proposition, although there remains room for doubt. One reason is that our cores do not penetrate into the glacial proper. Another is that the foraminiferal signals (both species distributions and stable isotopes) are strongly influenced by changing preservation of calcareous shells, from glacial to Holocene time.

Organic carbon contents (Fig. 1) are in agreement with our hypothesis. They vary between 0.45 and 0.13%. There is a general tendency for the values to decrease with depth in the sediment as a result of diagenetic oxidation, although this trend is obscured by unusually high values in the lower portion of the core. Using Holocene values of carbon content only, we derive an equation for the rate of decay of the organic carbon, of the form

$$C_D = C_0 e^{-kD} \quad (1)$$

where the concentration at depth D is given by C_D and the initial concentration is C_0 . The best-fit coefficient is $k = 0.0205$. The surface value was omitted before calculating the fit. The corresponding half life of decay is 12,000 yr. This is shorter than those found by Müller and Mangini²⁶ for regions of lower sedimentation rates. Superimposing the fit of equation (1) on the actual distribution of C_{org} accentuates the unusually high surface value, as well as the high pre-Holocene values (Fig. 1).

The pre-Holocene increase of C_{org} is familiar from hemipelagic sediments²⁷ and is presumed to be due to a greater nutrient input to the euphotic zone due to high intensity of coastal ocean mixing during glacial time. An analogous phenomenon occurs in the east equatorial Pacific²⁴. The change in productivity presumably is proportional to the change in content of organic carbon²⁷⁻²⁹. In the pre-Holocene sediment, we expect values near 0.15%, on the basis of the general trend, but we find values between 0.22% and 0.27%. An excess 0.07–0.12% C_{org} represents 47%–80% over background. Thus, we suggest that productivity dropped by a factor of 1.5–1.8 during deglaciation in this area.

The foraminiferal record is rather ambiguous. Both glacial and Holocene assemblages are dominated by *Neogloboquadrina dutertrei* (Dut in Fig. 2). This dominance increases upwards, apparently as a result of differential dissolution (note the increase in fragmentation of shells). There are two preservation maxima, one at the deglaciation-midpoint (as expected²³), and one centred on the Altithermal period in the early Holocene (A-max, Fig. 2). Several of our cores show a preservation maximum in approximately this position. The large changes in foraminiferal preservation make it difficult to extract information on the history of fertility. The decrease in abundance of the *Globorotalia menardii-tumida* complex (Men + Tum in Fig. 2) does suggest a fertility decrease. These forms are tied to tropical upwelling, and are dissolution-resistant.

The stable isotope data (Fig. 2) of three planktonic species show coherent trends. Taken at face value the increase in distance between $\delta^{18}O$ values of *N. dutertrei* and *Pulleniatina obliquiloculata* on the one hand, and *Globigerinoides sacculifer* on the other suggests an increase in thermal stratification, and hence a decrease in fertility. The effect of partial dissolution of $\delta^{18}O$ -values^{30,31} is in the wrong direction to supply explanations for the observed divergence. $\delta^{13}C$ -values have been linked to upwelling and fertility³²⁻³⁴. No convincing trend is seen in our data, although the lighter values below 30 cm would be in agreement with a higher productivity in pre-Holocene time. The effect of partial dissolution is to make values too light in the more highly dissolved Holocene section³⁰.

The phenomenon of the deglaciation fertility drop may be widespread. A deglacial Mn-rich layer exists in the west equatorial Pacific (photos in ref. 35, and unpublished analyses). In the central tropical Atlantic, a manganese-spike exists in approximately the correct position for deglaciation^{16,36,37}. (However, a steady-state origin has been invoked for this layer¹⁶.) There is a glacial–Holocene Fe-rich layer in the west-equatorial Atlantic³⁸. The stratigraphy of intensity of oxidation may provide important clues to the changing fertility of the ocean³⁹.

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Olivine to spinel transformation in Mg_2SiO_4 via faulted structures

J. N. Boland

State University of Utrecht, Institute of Earth Sciences,
PO Box 80.021, 3508 TA Utrecht, The Netherlands

Lin-gun Liu

Research School of Earth Sciences, Australian National University,
PO Box 4, Canberra, ACT 2600, Australia

The mechanism of the olivine to spinel transformation has been studied in relatively few systems experimentally, most of the previous research being directed to the determination of the phase boundaries in pressure–temperature (P , T) space¹ or crystal structure modelling of the low- and high-pressure phases²⁻⁶. Two of the more recent studies on magnesium germanate⁷ (Mg_2GeO_4) and nickel silicate⁸ (Ni_2SiO_4) revealed that there was no special orientation relationship between the olivine (α) and the spinel (γ). Furthermore, no evidence by way of stacking faults or twins was found in the transforming olivine to support the proposal that the transformation was martensitic-like⁴⁻⁶. Consequently, it was concluded that in these systems the transformation had occurred by nucleation and growth processes^{7,8}. In contrast, the α and γ phases have been observed as tabular intergrowths in the partially transformed iron end-member, fayalite (Fe_2SiO_4)⁹, with the interphase boundaries being $(100)_{O_i}$ or $(111)_{Sp}$. This microstructure and the orientation relationship are compatible with the martensitic transformation. We report here the results of transmission electron microscopy of the $\alpha \rightarrow \gamma$ transformation in the pure end-member olivine, forsterite (Mg_2SiO_4). Not only is there a special orientation relationship between the two phases during the transformation, that is, $(100)_{O_i}$ is parallel to $(111)_{Sp}$ and $[001]_{O_i}$ parallel to $[110]_{Sp}$, but some of the residual olivine grains have an extremely high density of stacking faults in $(100)_{O_i}$ as well as a high dislocation density.

The starting material consisted of synthetic olivine (Mg_2SiO_4 from TEM-Pres) and a minute amount of graphite. The latter was added for absorbing the laser and thus heating the sample. The starting material was compressed in a diamond-anvil cell to about 220 kbar over a 20-min period, and the temperature was then raised to $\sim 1,000^\circ C$ for 15 min by laser heating. Because of the pressure gradient in the cell, only the central region of the sample, which was subjected to the above specified pressure, was heated by the laser. The laser was then switched off (resulting in a rapid quench to room temperature) and the pressure was released over a 30-min interval. The central region (about 50 μm) of the quenched sample was ion-thinned and examined in the electron microscope. In the P , T conditions of the run, the spinel modification is thought to be the stable phase but in fact several residual olivine grains were detected, representing less than 1% of the sample under examination.

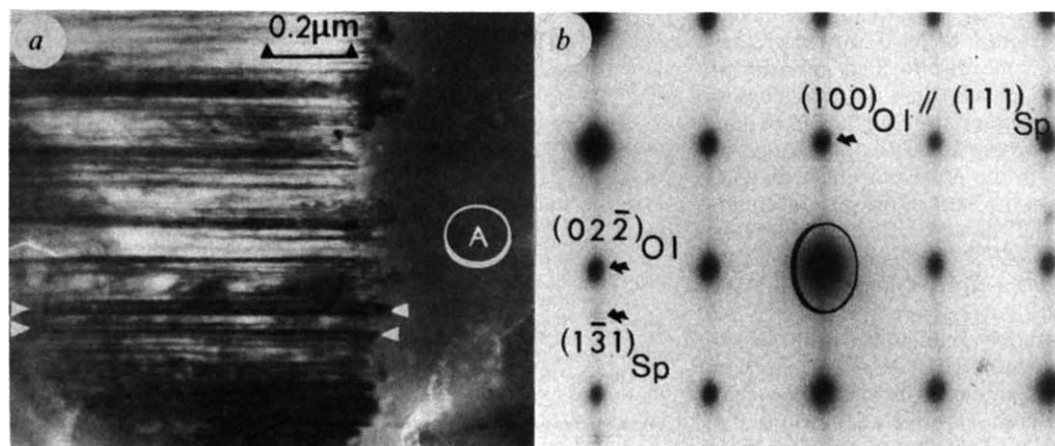


Fig. 1 *a*, The intercalated microstructure of partially transformed olivine grain on the left. The dark lamellae are the spinel phase in the olivine matrix that has a high density of dislocations. The region marked 'A' to the right was originally fine-grained spinel but it rapidly damaged in the electron beam to form an amorphous material. *b*, The composite diffraction pattern of the residual olivine in *a*. The olivine (Ol) and spinel (Sp) patterns are indexed. The orientation relationship between the two phases is $(100)_{\text{Ol}} \parallel (111)_{\text{Sp}}$, $[001]_{\text{Ol}} \parallel [\bar{1}10]_{\text{Sp}}$.

Residual olivine was also confirmed by an X-ray powder diffraction study of the bulk-heated area of the sample before thinning. These residual olivine grains have apparently resulted from incomplete heating (or incomplete transformation) in the experiment.

The residual olivine contained an extremely high density of dislocations ($>10^{12} \text{ cm}^{-2}$) as well as planar defects. An appropriately oriented grain is shown in Fig. 1*a*. The corresponding diffraction pattern in Fig. 1*b* shows the olivine pattern but superimposed on it are: (1) a second discrete single-crystal-like pattern that was indexed according to the spinel structure and (2) continuous streaking in the diffraction pattern parallel to $(100)_{\text{Ol}}$ or $(111)_{\text{Sp}}$. The orientation relationship is shown in Fig. 1 for the setting $Pbnm$.

It is clear from Fig. 1 that the olivine and the spinel are intercalated. One may interpret this transformational growth pattern in terms of the model proposed for a martensitic transformation⁴⁻⁶ in which dislocations with Burgers vector $[001]$ dissociate while gliding on $(100)_{\text{Ol}}$, the plane corresponding to the hexagonal close-packed (h.c.p.) planes of the oxygen sublattice. The dissociation proposed is: $[001] \rightarrow 2 \times (1/12)[013] + 1/12[0\bar{1}3]$ in which only the propagation of the $1/12[013]$ partials produces the required h.c.p. to c.c.p. (cubic close-packing) of the oxygen sublattice required for the spinel structure. The cations rearrange themselves by a synchroshear process. The major difficulty with this model has been the failure to observe dislocation dissociation to any considerable extent in olivine and even in the reported observations¹⁰ of actual split dislocations, represented by images of 40 Å separation, the interpretation has to be seriously questioned because the diffraction conditions did not conform to the stringent conditions necessary for the correct interpretation of weak beam images¹¹. The high density of dislocations in our sample prohibited good weak beam images but diffraction studies of the image contrast at the dislocations¹² showed that most of the Burgers vectors were $[100]$ with some $[001]$ (10^8 cm^{-2}) and a minor amount of $[010]$ —the last type, although having a rather large length of 10.2 Å, has also been reported in Ni-olivines subjected to high pressures⁸.

The P , T conditions inside the diamond-anvil cell of the present experiment are difficult to specify accurately but there are large temperature and pressure gradients, the latter adding the non-hydrostatic stress condition most probably contributing to the excessively high content of dislocations observed in the olivine.

The effects of pressure gradients were also reported in diamond-anvil runs on Fe_2SiO_4 . The P , T conditions used in the Mg_2GeO_4 (ref. 7) and Ni_2SiO_4 (ref. 8) runs may be better defined, although non-hydrostatic effects may still be important. However, the ability to quench extremely rapidly in the diamond-anvil experiments (10^6 °C s^{-1} according to ref. 9) may allow more faithful sampling of the phase assemblages at the P , T conditions, provided quench phases do not appear⁷. In

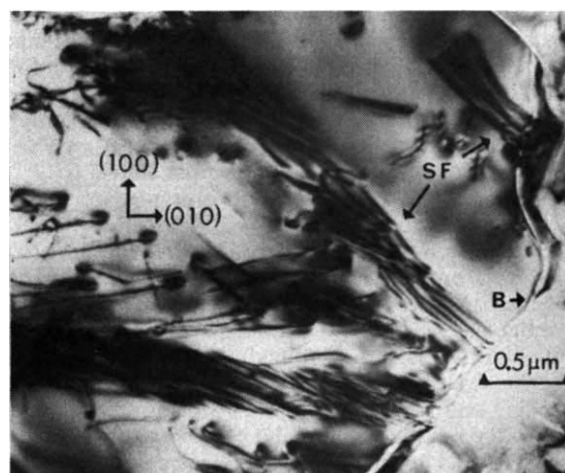


Fig. 2 Spinel (Mg_2SiO_4) with its stacking fault fringes, SF, on $\{110\}$ and dislocation arrays. The grain boundary at B is fractured.

our run, only two grains of β phase were identified; their microstructure was similar to that reported in the Tenham chondritic meteorite (Fig. 2 of ref. 13). The infrequent occurrence of the β phase is related to the rapid transit through its phase field so that the $\alpha \rightarrow \gamma$ transformation may occur without the intermediate β -form, although the (100) planar faults in the olivine are important for the transformation.

The quenched, metastable spinel phase does not have long-term stability in the electron beam and may transform to an amorphous phase; this is especially evident in the vicinity of the olivine–spinel interphase boundary as shown in Fig. 1*a*. The boundary is notably stepped and the high dislocation density makes it difficult to obtain clear images of the defect structures. Away from the boundary regions the spinel grains can be imaged to reveal their defect contents. Their grain size ranges from 0.25 to 5.0 μm in exceptional cases. All the grain boundaries are fractured, as previously noted in the Ni_2SiO_4 spinel⁸, and most of the dislocations within the grains, have stacking faults associated with them (Fig. 2).

The behaviour of forsterite differs from that of the other olivine structures in that it passes through a β -phase field in the pressure range 140–200 kbar while the olivine phases of Mg_2GeO_4 , Ni_2SiO_4 and Fe_2SiO_4 transform directly to the γ phase. The uniqueness of the β phase has been re-examined recently³ in a study of the crystal-structure modelling in the system Ni_2SiO_4 – NiAl_2O_4 . Starting with the spinel structure (symbol S) as one of the endmembers, it is possible to generate a number of the observed intermediate phases by introducing planar boundaries (T) at regular intervals into the (110) stacking sequence. The boundaries may be described either as anti-phase

boundaries with a displacement vector $1/4[112]_{sp}$, or reflection/twin planes. The different structures are generated by periodic operation of T. The β phase is symbolized by (TS)², being only an intermediate phase between S and the other end-member T—the hypothetical ω phase. It is this ω phase that has been postulated as the linking phase to the spinel series and the mechanism for its formation is almost exactly that proposed earlier for the martensitic transformation⁴⁻⁶, except the new mechanism suggests a two-step shearing, one of the cation array and the other of the anion array, by the passage of $1/12[013]$ partial dislocations across alternate anion layers.

The streaking in the diffraction pattern along $(100)_{Ol}$ indicates that there are many stacking faults in that plane and that the

transition from α to γ occurs by the glide of partial dislocations, some of which produce sufficiently wide slabs of the spinel phase to suggest cooperative motion of the dislocations akin to what is observed in metallic systems¹⁴. There is no evidence of the ω phase but the olivine to spinel transformation in Mg_2SiO_4 proceeds via an intermediate stage of faulting (maybe unit-cell slabs of ω phase or other members of the spinelloid group).

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Uranium enrichment in Archaean crustal basement associated with overthrusting

L. M. Lobato & J. M. A. Forman

Nuclebrás, Rio de Janeiro, Brazil

W. S. Fyfe, R. Kerrich & R. L. Barnett

Department of Geology, University of Western Ontario, London, Ontario, Canada N6A 5B7

Uranium deposits cumulatively in the 100,000 tonne U_3O_8 range occur within ductile shear zones transecting Archaean basement gneisses at Lagoa Real, north-east of Caetité, State of Bahia, Brazil¹⁻³ (Fig. 1). The gneisses, dated at 2,600-3,000 Myr, are at amphibolite to granulite facies of metamorphism. There is a general consensus that such rocks form near the base of the crust, and typically exhibit pronounced depletion of U relative to the upper crust^{4,6}. To the east and west of Lagoa Real the Archaean gneisses overlie Proterozoic meta sediments and volcanics of the Espinhaço supergroup along well documented thrust surfaces^{7,8}. Limited U-Pb dating of the deposits indicates that U-mineralization occurred at ~820 Myr (ref. 9), involving removal of Si and K coupled with Na addition in oxidizing conditions. V, Pb and light rare-earth elements (LREE) were introduced along with uranium, from hypersaline CO_2 -hydrocarbon-rich fluids, with $\delta^{18}O$ -4‰ at temperatures of ~540 °C, depths of ~15 km, and $P_{fluid} > P_{load}$. We show here that the geological constraints are consistent with release, through hydrofracturing, of ~1,000 km³ of overpressurized-meteoric water-recharged formation brines in the Proterozoic sediments up through the hot overriding gneisses.

In proximity to domains of U-mineralization hydrothermal metasomatism has overprinted the regional amphibolite grade metamorphism of the gneisses on a massive scale, sporadically 40 km across and 100 km along strike. Albite plagioclase is the dominant mineral in 'ore grade' zones; uraninite is present up to 2%. The principal mineral reactions accompanying introduction of U are: (1) K-feldspar is altered to albite, (2) hornblende is replaced by ferro-hastingsite, (3) sphene, magnetite and aegirine-augite are formed, (4) quartz is dissolved, and (5) haematite, calcite, andradite garnet, and epidote-allanite appear as late phases. Overall, the alteration has involved massive enrichment of Na and Fe^{3+} , with a concomitant deple-

tion of K and Si. Removal of silica through solution of quartz requires aqueous fluids to travel up thermal gradient, and oxidation could then be in response to the mechanism discussed by Beach and Fyfe¹⁰.

Constraints on pressure are sparse. Kyanite coexisting with muscovite in metarhyolites of the Espinhaço supergroup signifies pressures of >3 kbar at temperatures of <650 °C (refs 11, 12). Higher pressures are unlikely in view of the low jadeite content of the pyroxenes¹³. U orebodies transect the ductile shear zone fabric, and are locally caught up in renewed movement along these structures. These relations signify that the shear zone acted as conduits of high permeability for release of an overpressured reservoir, through hydrofracturing, in conditions where $P_{fluid} > \sigma_3 + I(\sigma_3)$, minimum confining stress; T , tensile strength of gneiss), during episodes of transient switching in the principal stress orientations across shear zones¹⁴.

Uranium thorium ratios are extremely high, and while U averages 0.15%, thorium never exceeds 10 p.p.m., a value commensurate with Th abundances in average felsic gneisses. Several elements are enriched along with U in the shear zones including V (mean 590 p.p.m. $\pm$ 360 1 σ), Pb (460 $\pm$ 316 1 σ), La (1,530 $\pm$ 1,140 1 σ) and Ce (546 $\pm$ 230 1 σ)⁹.

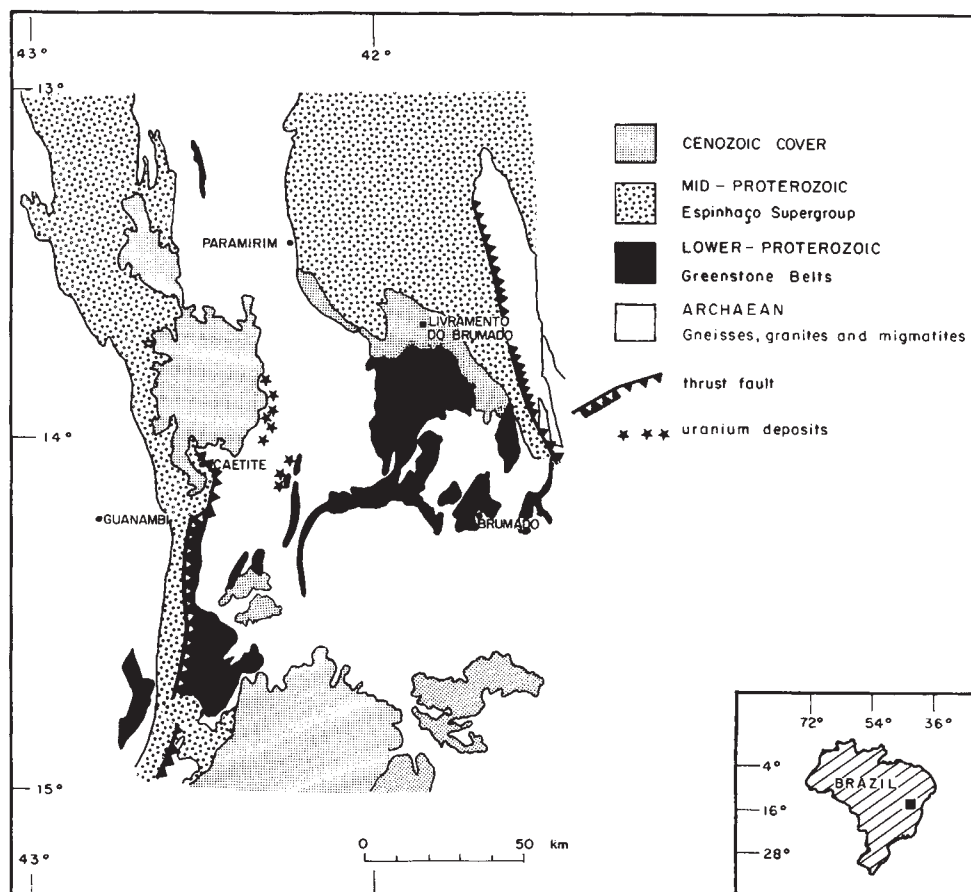
Systematic variations in the mineral chemistry of clinopyroxenes has been recorded across the domains of U mineralization. Within gneisses, pyroxenes are unzoned, chemically uniform and relatively magnesian, with FeO/MgO in the range 0.80-1.0. Although minor Na and Al substitution is present, these minerals are essentially members of the diopside-hedenbergite series. In proximity to U-ore pyroxenes are zoned from Mg-cores to Na and Fe^{3+} -rich rims, such that FeO/MgO rises to 4.5 in the margins of some grains and in high grade ore pyroxenes average 10 wt% Na_2O with FeO/MgO ~9.6 (Fig. 2).

Whole rock δ -values for the gneisses range from 7.2 to 10.2‰ (Table 1). The highest values are isotopically heavy compared with the $\delta^{18}O$ for primary igneous rocks of granodioritic composition^{15,16}, signifying either low-temperature hydrothermal alteration of a granodiorite precursor, or alternatively a high ^{18}O component to the gneissic parental rocks. At shear zone boundaries (sample 04) quartz, oligoclase and magnetite are triply concordant, signifying a deformation temperature of 500-540 °C, in the presence of fluids with $\delta^{18}O$ +5.8 to +6.4‰—probably metamorphic fluids indigenous to the host gneisses.

Within U-mineralized shear zones quartz, feldspar, pyroxene, and magnetite all undergo a shift of about -10‰ relative to their δ -values in the parental gneiss (Fig. 3). Quartz-magnetite fractionations are in a narrow interval of 8.3-9.0‰, corresponding to isotopic temperatures of 500-540 °C. In samples M1 and

Table 1 The oxygen isotope composition of mineral separates and whole rocks from country rock gneiss, and uranium mineralized counterparts within shear zones

Sample description	Sample no.	$\delta^{18}\text{O}$ quartz	$\delta^{18}\text{O}$ feldspar	$\delta^{18}\text{O}$ pyroxene	$\delta^{18}\text{O}$ magnetite	$\delta^{18}\text{O}$ whole rock	Δ (quartz-feldspar)	$\Delta^{18}\text{O}$ (quartz-pyroxene)	$\Delta^{18}\text{O}$ (quartz-magnetite)	Temperature (°C)	$\delta^{18}\text{O}$ fluid
Gneiss											
Quartz, oligoclase,	0.1	12.1	9.8*		2.5	10.1	2.3		9.6		
K-feldspar, pyroxene,	0.2	10.9	8.5		1.0		2.4		9.8		
biotite $\pm$ magnetite	0.3	11.3	8.6		1.2	8.3	2.7		10.2		
	0.4	8.8	7.0	6.04	0.4		1.8	2.7	8.4		+6
	0.5	9.0	7.2			7.2	1.9				
	0.6	9.9	7.7				2.2				
Zone of U-mineralization											
Albite, pyroxene,	M1	-1.2	-2.6†		-9.5		1.4		8.3	540	-4
quartz, magnetite	M2	-0.5	-1.4		-8.4		1.9		9.0	500	-3
	M3	-0.0	-1.7	-4.15	-8.7		1.7	4.1	8.7	520	-3

* $\delta^{18}\text{O}$ of oligoclase in the gneiss.† $\delta^{18}\text{O}$ of albite in uraniferous rocks.**Fig. 1** Simplified geological map of the state of Bahia, Brazil, illustrating the disposition of Archaean gneisses, lower Proterozoic greenstone belts, the upper Proterozoic Espinhaço Supergroup sedimentary sequence, thrusts and uranium deposits (after ref. 4).

M2 coexisting quartz, albite, and magnetite are triply concordant, and in sample M3 quartz, albite, pyroxene and magnetite are quadruply concordant, providing a rigorous criterion for retention of isotopic equilibrium in the mineralized rocks¹⁷⁻²¹. Errors in temperatures calculated from quartz-magnetite fractionations amount to about $\pm 10^\circ\text{C}$.

Fluids implicated in the uranium mineralization have a $\delta^{18}\text{O}$ of -3 to -4% , an isotopic signature consistent only with meteoric water¹⁶. Fluid inclusion data on late stage calcites reveals hypersaline, hydrocarbon and CO_2 -rich fluids within the ore zones²², although this does not necessarily apply to the U-transporting fluids. The U-bearing fluids are interpreted to have been derived by dewatering of formation brines that originated from meteoric water recharge of the Espinhaço sedimentary aquifers. Modern formation brines such as in the Illinois and Alberta sedimentary basins possess a low $\delta^{18}\text{O}$ that

reflects the isotopic composition of ambient continental meteoric water recharge¹⁶.

Any viable hypothesis for the origin of the uranium deposits in high-grade gneisses must account for the following geological and geochemical constraints.

- (1) Fluids capable of carrying significant U, V, Pb and REE.
- (2) A fluid reservoir characterized by high Na/K, and low ^{18}O , that is not indigenous to the host gneisses (and possibly of high salinity and with abundant hydrocarbons).
- (3) Discharge of the fluid reservoir in conditions of $P_t > \sigma_3 + T$, with fluids moving up thermal gradient to $\sim 540^\circ\text{C}$, and inducing oxidation.
- (4) Mineralization proceeding at depths of ~ 15 km.
- (5) Mineralization located within ductile shear zones in older high-grade gneisses in thrust contact above younger rocks of lower metamorphic grade.

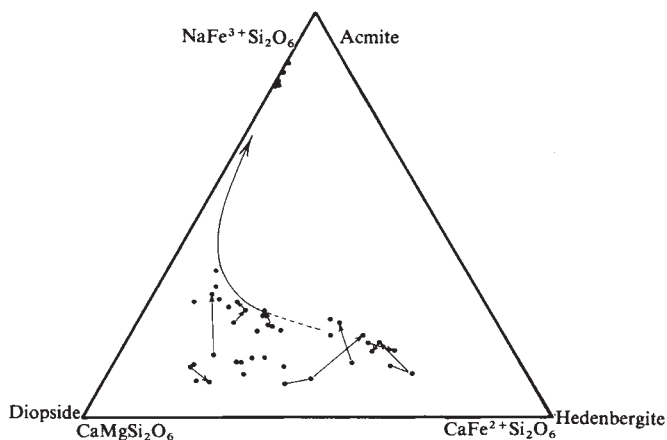


Fig. 2 Chemical variation of clinopyroxenes across shear zones at Lagoa Real. Small arrows signify core-rim zonation, large arrow indicates the overall trend of composition towards the highest state of alteration.

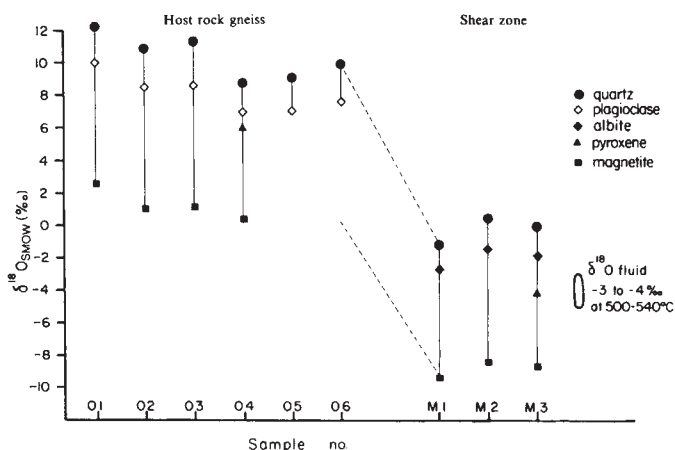


Fig. 3 Oxygen isotope composition of mineral separates from gneisses and their deformed U-mineralized counterparts, at Lagoa Real, Brazil.

A possible model for the U mineralization arises from consideration of the regional structure of the area. The Archaean basement is either faulted up or thrust over the Proterozoic sediments 30 km to the west (Fig. 1)^{7,8}, and this structure appears to be common for something like 600 km along a north-south trend in Bahia and south into the state of Minas Gerais. Uranium anomalies are parallel to this regional structure, and granulites over wide regions of the State of Bahia have anomalous geochemistry in terms of lack of LIL depletion, and so on^{23,24}. Increasingly, modern seismic studies of continental crustal structure show that thrust tectonics of various types is ubiquitous²⁵⁻²⁹.

If we examine such a thrust model for the Lagoa Real district the tectonic-magmatic development might be as follows: First, updoming of the crust occurred to the East probably by a thermal high, with updomed basement detached and sliding to the west (see ref. 30). Gabbroic-granitic volcanism and plutonism have been recorded over the period 1,100-400 Myr in this general region⁴, along with granites at 820 Myr, a similar age to that deduced for the uranium mineralization. Second, the gravity driven thrust slices must have had basal temperatures close to the melting temperatures of granite suggesting that partial melting triggered the thrusting (see ref. 30). Such tem-

peratures are required to explain the inverted metamorphic gradient in the underthrust Espinhaço at the thrust boundary.

Finally, loading coupled with a temperature rise in the Espinhaço sediments arising from the hot overthrust gneisses would induce dehydration and overpressure aquifers causing high fluid pressures necessary to maintain thrust motion³¹. Such high-pressure fluids would penetrate up through the basement by hydraulic fracture with $P_{\text{fluid}} \geq P_{\text{lithostatic}} + T$.

Regions of fluid penetration by hydraulic fracture mechanisms would be confined to zones of weakness in the over thrust plate (shear zones) which themselves could be related to topography of the thrust plates. This proposed fluid source for the uranium deposits assumes meteoric water recharge of the Espinhaço sedimentary rocks, a well documented feature of contemporary hydrology¹⁶, and retention of much of the water during overthrusting, before discharge through the shear zones. Downwards, hydrostatic penetration of oxidized, saline and U-bearing meteoric water to temperatures and depths of 500 °C, 15 km could account for the observed silica removal, oxidation, and low ¹⁸O fluids, but this hydrological regime is not consistent with the high fluid pressures inferred for shear zones and required for thrusting.

Based on an upper limit of ~100 parts per 10⁹ for U solute concentrations in deep groundwaters^{32,33}, in excess of 1,000 km³ of hydrothermal solutions would be required to account for the 100 ktonne of U present in the shear zones. Release of 5% pore water alone from a lower grade under plate 5 km thick would readily account for such a fluid volume. The mechanism of U, V, Pb and REE transport is not resolved.

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Organic components in historical non-metallic seals identified using ^{13}C -NMR spectroscopy

M. Cassar, G. V. Robins, R. A. Fletton* & A. Alstin†

Institute of Archaeology, University of London, 31–34, Gordon Square, London WC1H 0PY, UK

* Glaxo Group Research Ltd, Greenford Road, Greenford, Middlesex UB6 HE, UK

† Conservation Department, Public Records Office, Chancery Lane, London WC2, UK

Seals attached to archival documents have considerable historical interest, not least from the insight that their composition and construction allows into early technological practices. Since the mediaeval period many non-metallic seals have been stored in collections in Britain¹ and their deterioration is a cause of continual concern. Our interest in the composition of these seals arises out of this concern and the general archival philosophy of using materials that closely match the original components for repair work. We report here the first application of ^{13}C -Fourier transform NMR (FTNMR) spectroscopy to the determination of organic materials in this kind of archaeological or historical context. The organic components of a small number of historical seals have been examined and the chemical stability of the components observed by comparison with modern and ancient materials suspected to be in the seals. We suggest that ^{13}C -FTNMR can be applied widely for identification purposes in such areas involving the conservation of historical and archaeological organic materials.

There has been only one earlier paper, by Dobbie and Fox² in 1914, on the analysis of these materials, although a recent study of resins and waxes in a related area with gas chromatography—mass spectrometry³ is of some relevance. This paucity of data is relieved, however, in the historical and contemporary literature^{4,5} where recipes and formulations are suggested for seal construction. Generally, the organic component is recommended as a wax: resin mixture in 2:1 proportions, to which is added an inorganic pigment—mercury(II) sulphide as cinnabar in red seals, and copper(II) acetate as verdigris in green seals. In addition, fillers of chalk, flour or other material might be incorporated in the construction of complex layered seals.

Our analytical interest was centred on the examination of the organic constituents both from the viewpoint of component identification and the assessment of their deterioration. We also examined samples of the seals with X-ray fluorescence and X-ray diffraction to determine the nature of the pigments and fillers; these findings will be discussed elsewhere. Among the comprehensive series of seals made available for study we selected three Royal seals—those of Kings Stephen (1135–54), John (1199–1216) and William IV (1830–37)—and one unprovenienced personal mediaeval seal, and compared these with both ancient (*circa* fourteenth century) and modern beeswax, which is the most commonly specified historical seal wax, and with colophony (rosin) and shellac, which were anticipated to be resin components in some of the seals.

Initial studies of the modern comparison materials by IR spectroscopy, using both melt- and solvent-deposited films, showed that mixtures would be difficult to resolve spectroscopically because of the complexity and similarity of the components. We decided, therefore, to utilize ^{13}C -FTNMR for the analyses since this technique offered the possibility of distinguishing between suspected components. The chemical shifts⁶ of the long saturated chains anticipated in the waxes⁷ should be considerably different from the chemical shifts of the cycloalkenyl moieties which have been suggested as structural units

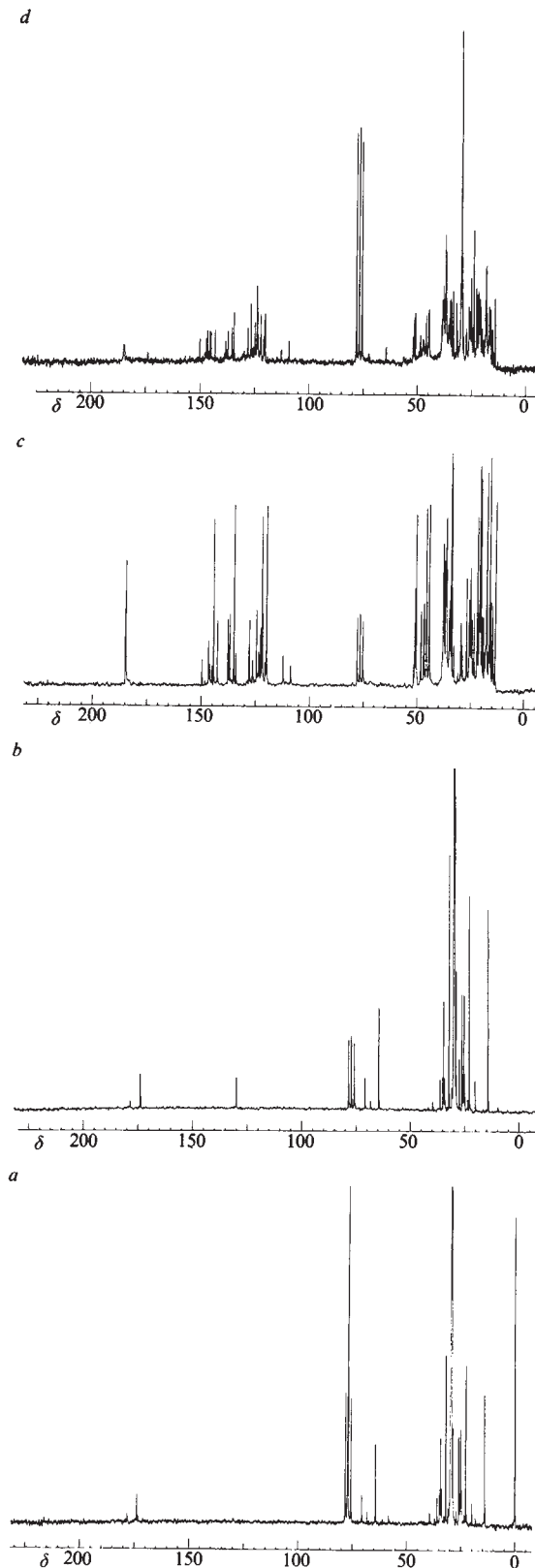


Fig. 1 *a*, Beeswax *circa* fourteenth century. *b*, Beeswax, modern and unbleached. *c*, Colophony (rosin), modern. *d*, Royal Seal of William IV. All spectra were recorded at 25.05 MHz with full proton decoupling using a Jeol FX100 FTNMR spectrometer, with a 6-kHz spectrum width, 35° pulse width and a pulse repetition time of 0.8 s. Before Fourier transformation free induction decays were zero filled to 16 K data points and exponentially weighted to give a line broadening of 0.8 Hz.

in the resins⁸ from the observation that they can be degraded into abietic acid units.

¹³C-FTNMR spectra are shown in Fig. 1, where the solvent, deuteriochloroform, gives rise to a triplet at $\sim\delta 77$ and the tetramethylsilane reference, where present, gives a singlet at $\delta 0$. The singlet at $\delta 77$ in Fig. 1a arises from residual chloroform used in the preliminary extraction of the sample. In modern beeswax (Fig. 1b) we observe a characteristic fingerprint in the alkane region ($\delta 20$ – 40) with weak signals from alkene or aromatic carbons showing at $\sim\delta 130$ and ester carbonyl signals in evidence in the region $\delta 170$ – 180 . These spectral features were common to samples of beeswax from other contemporary sources and we noted that the *circa* fourteenth century beeswax (Fig. 1a) showed a comparable spectrum except in the region around $\delta 130$ where loss of unsaturation through oxidation is the most likely explanation. Solutions of the King Stephen and King John seals, and the mediaeval personal seal also showed the beeswax spectrum almost unaltered from that of the modern material; a feature which strikingly confirms the contention of Dobbie and Fox that the waxes possess long-term chemical and microbial stability and that seals are most vulnerable through microbial attack on the layered filler where present. The presence of mercury and copper compounds must also be linked

with microbial stability of the organic compounds that act as their dispersants.

The later seal of William IV, however, showed a more complex spectrum (Fig. 1d). This, we suggest, shows evidence in the alkane and ester carbonyl regions for the presence of beeswax and in the $\delta 130$ – 150 region for a resin component similar to but not necessarily identical with modern colophony (Fig. 1c)—the anticipated resinous component. While the spectral features are incompatible with shellac, it is possible that this is deteriorated colophony and we hope to resolve this question during further studies of these materials. It is evident that ¹³C-FTNMR is a useful diagnostic tool for distinguishing between the components of such organic mixtures and we suggest that it might be used widely in such problems in conservation where the solubility of the target material allows.

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Decontamination of *Bacillus anthracis* on Gruinard Island?

R. J. Manchee, M. G. Broster, Isabel S. Anderson & R. M. Henstridge

Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK

J. Melling

Public Health Laboratory Service, Vaccine Research and Production Laboratory, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG, UK

In experiments on Gruinard Island 40 years ago small bombs containing spores of *Bacillus anthracis* were suspended from a gantry and detonated, producing widespread contamination of the island's surface. Recently, analysis of soil samples has shown that the area where the spores can now be detected is small enough to be considered for decontamination¹. We investigated the effect of treating the soil with sporicidal chemicals, namely, potassium permanganate, formaldehyde, glutaraldehyde, Cidex ('activated' glutaraldehyde, Surgikos), dodecylamine and peracetic acid.

Initial laboratory tests showed that formaldehyde (2%) and glutaraldehyde (2%) were more effective than dodecylamine (1%) in killing spores of *B. anthracis* in suspension and spores of *Bacillus subtilis* var. *niger* in beds of peat 30 cm deep but none of the chemicals sterilized the peat beds at the concentrations used. Peracetic acid was not included in the laboratory tests because of its known lethal effect on spores of *B. anthracis*². The chemicals chosen for trials on Gruinard Island were formaldehyde, glutaraldehyde, peracetic acid, and dodecylamine, which was included for its unusual mode of action³ and low toxicity to vegetation, although it had not performed well in the laboratory tests.

Table 1 Recovery of viable spores of *B. anthracis* from Gruinard soil treated with disinfectants

Treatment	Soil depth (cm)	No. of spores recovered before and after treatment with disinfectants			
		Enclosed plots		Open ground	
		Before	After	Before	After
Glutaraldehyde (5%, 500 ml)	0–2	105	0	0	0
	2–4	255	18	0	3
	4–6	30	3	3	—
	6–8	0	—	—	—
	>8	0	—	—	—
Glutaraldehyde (5%, 1,000 ml)	0–2	9	0	66	No sample recovered
	2–4	354	0	21	
	4–6	75	—	3	
	6–8	9	—	—	
	>8	—	—	—	
Formaldehyde (5%, 500 ml)	0–2	0	0	0	0
	2–4	531	0	105	0
	4–6	231	0	3	—
	6–8	6	—	—	—
	>8	3	—	—	—
Formaldehyde (5%, 1,000 ml)	0–2	51	0	0	0
	2–4	396	0	—	0
	4–6	90	0	—	0
	6–8	12	—	—	—
	>8	0	—	—	—
Peracetic acid (3%, 1,000 ml)	0–2	357	0	66	0
	2–4	102	—	96	—
	4–6	24	—	—	—
	6–8	9	—	—	—
	>8	21	—	—	—
Dodecylamine (0.1%, 1,000 ml)	0–2	39	312	3	216
	2–4	3,150	8,010	1,312	183
	4–6	10,700	—	21	6
	6–8	141	—	9	3
	>8	300	—	9	0

In samples where *B. anthracis* content was low or absent, the sporicidal effect was assessed by examination of the other soil microflora. Glutaraldehyde, formaldehyde and peracetic acid eliminated or greatly reduced this flora but dodecylamine did not. —, Core did not extend to this depth.

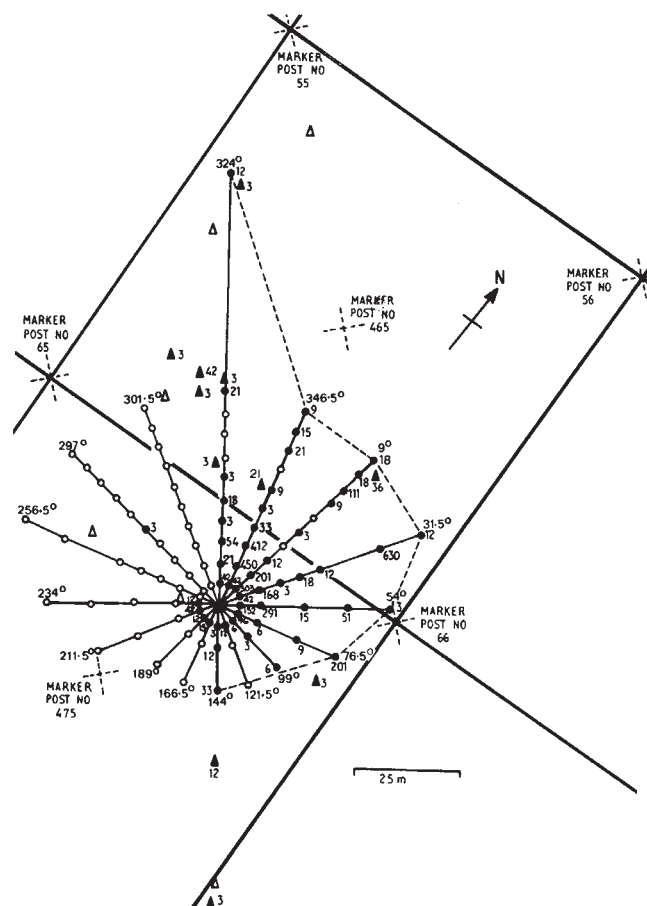


Fig. 1 The origin of the sampling array is the release point gantry. An initial compass bearing was taken on a line of old marker posts (324°) and the remaining lines were drawn at 22.5° intervals: the compass bearing is shown at the end of each line of samples. The array is shown in relation to the 1979 sampling grid¹ (large squares). Symbols: ●, positive sample from array; ○, negative sample from array; ▲, positive sample from pool or rill; △, negative sample from pool or rill. The numbers adjacent to the symbols for positive samples indicate the number of spores of *B. anthracis* per gramme of soil isolated from the top 8 cm of each core sample.

The experimental plots were in an area of known contamination a few metres north of the gantry. Enclosed plots were constructed by cutting the vegetation to soil level and driving pieces of PVC pipe (15 cm i.d.) into the soil to a depth of about 30 cm. Open plots were areas of unprepared ground about 15 cm in diameter. Chemicals were diluted to working strength in seawater, except dodecylamine which was dissolved in tap-water containing 0.1% ethanol. The plots were sampled before the chemicals were applied and 10 days after application: a further 21 days elapsed before the cores were examined using Knisely's selective culture medium⁴. Only dodecylamine failed to kill the spores in the soil (Table 1). There was little difference between enclosed and open plots. Formaldehyde (5%) has been chosen for larger scale trials because it is effective and relatively cheap.

Analysis of soil samples taken in a radiating pattern with the gantry as the point of origin showed that the area of detectable contamination is located between 300° and 166° in a clockwise direction, but insufficient samples were taken to define its radius (Fig. 1). The almost complete circle of contamination 5 m from the gantry is probably due to splatter from the detonations. Generally the spores are distributed between north-west and north-east of the gantry and diminish in numbers with increasing distance. This pattern is probably due to the influence of wind at the time of the trials. Further samples will have to be taken to define the area requiring treatment. If large-scale decontamination is eventually decided upon careful consideration will

have to be given to the ecological effects of extensive use of nonselective biocides.

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Visual processing in the leech central nervous system

Eric L. Peterson

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada H3A 1B1

In animals with complex visual systems spatial contrast is enhanced by mutual inhibition between retinal neurones monitoring different fields¹. By analogy, if animals like leeches and some caterpillars, that have several simple (non-image-forming) eyes aimed in different directions, are capable of rudimentary form detection, one might predict mutual antagonism between eyes monitoring different fields. In support of this prediction, I report here a paired interneurone in the central nervous system (CNS) of the leech which is stimulated by eyes on one side of the animal and inhibited by eyes on the other. There are striking parallels between these neurones and other integrating neurones, in particular those processing bilateral auditory input in crickets², suggesting that the visual system of the leech may be representative of a general class of sensory processing systems.

Many aspects of the CNS of the leech, *Hirudo medicinalis*, are well understood³, but very little is known about how it processes visual information. The leech's visual system includes five pairs of eyes, numbered 1–5 in a front-to-rear sequence on the surface of the head (Fig. 1a), and numerous photosensitive sensilla⁴ (not discussed here). An eye consists of a pigmented eye-cup packed with about 50 randomly arranged photoreceptor cells⁵. There is no lens, no image, and no contacts between photoreceptors within the eye^{5,6}. Thus each photoreceptor apparently responds independently to light within a field of view defined by the simple geometry of the eye-cup. The optical axis of the eye is roughly perpendicular to the surface of the body wall and its angle of acceptance is about 75° (ref. 4), and although the relationship between the eyes varies as the leech changes shape, as a rule eyes on opposite sides of the head will have different fields of view.

Light depolarizes leech photoreceptors^{6–8} (Fig. 1b) and elicits impulses which evidently arise in the photoreceptors' axons^{4,6,8}. A spike in the soma is matched by an impulse entering the head-brain via the optic nerve 15–25 ms later (Fig. 1c). A search for the central targets of the photoreceptors revealed a bilateral pair of neurones, the LV (lateral visual) cells, in the anterior central packet of the first segmental ganglion. An LV cell receives a compound excitatory postsynaptic potential (e.p.s.p.), which is graded with light intensity, when the ipsilateral eyes 3, 4 or 5 (but not eyes 1 or 2) are illuminated. The photoreceptors apparently make direct electrical synapses with the LV cells, as single impulses in a photoreceptor produce small, fixed-latency e.p.s.ps (Fig. 2a) whose rise time (1.5 ms) is much shorter than that of known chemical e.p.s.ps in the leech CNS⁹. Moreover, the excitatory response persists in 40 mM Mg leech saline, a solution that blocks chemical synaptic transmission in the leech⁹. Given a long pulse of light (Fig. 2b) the LV cell, like the photoreceptors⁴, has a sharp 'on' response, rapidly adapts to a lower firing rate in maintained light, and has no 'off' response.

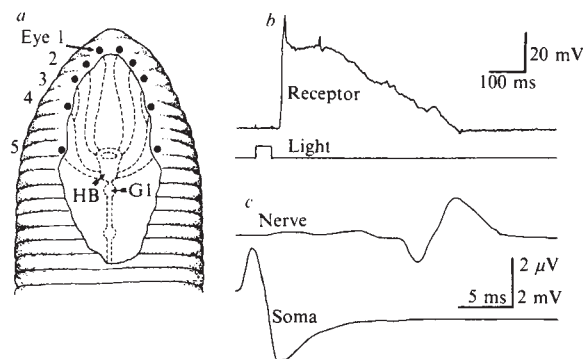


Fig. 1 *a*, Cutaway diagram of the front end of the leech, *Hirudo medicinalis*, showing the five pairs of eyes, the associated optic nerves and a portion of the CNS including the head-brain (HB) and the first segmental ganglion (G1). *b*, Lower trace: light monitor. The flash (approximately 10^4 erg cm^{-2} white light) was applied with an electronic shutter (Uniblitz). Upper trace: intracellular recording from the soma of a photoreceptor in eye 5. *c*, Lower trace: average of 90 spontaneous impulses recorded intracellularly from a photoreceptor in eye 5 in the dark. Upper trace: averaged simultaneous extracellular recording of the optic nerve of eye 5 taken near its point of entry into the head-brain.

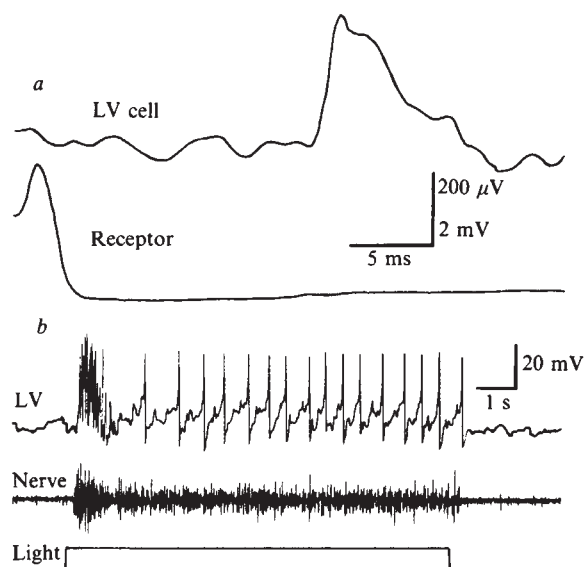


Fig. 2 *a*, Lower trace: average of 44 spontaneous impulses recorded intracellularly from a photoreceptor in eye 5 in the dark. Upper trace: averaged simultaneous intracellular recording of the LV cell, showing a unitary e.p.s.p. *b*, Lower trace: light monitor, conditions as in Fig. 1b. Middle trace: extracellular recording from the optic nerve of eye 5. Upper trace: intracellular recording from ipsilateral LV cell.

By contrast, when the contralateral eyes 3, 4 and 5 are illuminated, an LV cell receives a complex barrage of inhibitory postsynaptic potentials (i.p.s.ps) (Fig. 3) whose short, fixed latency suggests that there are few if any interneurons interposed between the contralateral receptors and the LV cell. The contralateral photoreceptors do not, however, inhibit an LV cell directly because treatments that preferentially block polysynaptic pathways in the leech (20 mM Ca; 20 mM Mg saline)⁹ block contralateral inhibition. Nor is the contralateral LV cell part of the inhibitory pathway, as impulses induced by injecting current into one LV cell do not produce i.p.s.ps in the other. Obviously there are other visual interneurons yet to be found.

LV cells injected with horseradish peroxidase and processed for light microscopy all had ipsilateral and contralateral processes in the first segmental ganglion, and an ascending contralateral process that terminated in the supra-oesophageal ganglion. In four of seven preparations (Fig. 4) there was also a fine process that ascended ipsilaterally a short distance into the

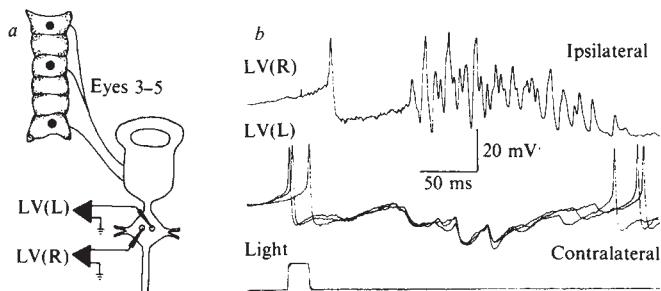


Fig. 3 *a*, LV cells recorded intracellularly in a preparation in which eyes 3-5 on one side only were attached. *b*, Lower trace: light monitor, conditions as in Fig. 1b. Middle trace: three superimposed responses from the contralateral LV cell. Upper trace: a single response from the ipsilateral LV cell. Impulses seen before, during and immediately after the light pulse are spontaneous.

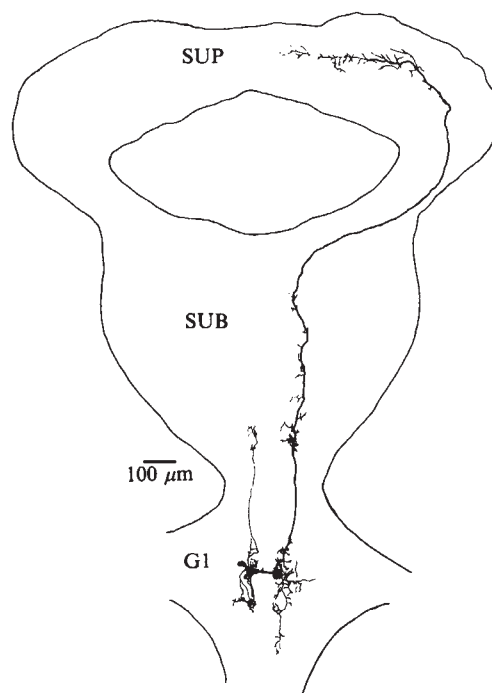


Fig. 4 Camera lucida drawing of a horseradish peroxidase-filled LV cell, developed with diaminobenzidine¹⁷ and viewed as a wholemount. Labels: SUP, supra-oesophageal ganglion; SUB, sub-oesophageal ganglion; G1, first segmental ganglion.

sub-oesophageal ganglion. Preparations stained with Lucifer yellow often showed in addition a broad bundle of many fine fibres which arose near the soma of the LV cell, then coursed anteriorly into the sub-oesophageal ganglion and exited through the nerves that lead to eyes 3, 4 and 5. These fibres may have been incoming axons of photoreceptors stained by transfer of Lucifer yellow across electrical junctions¹⁰ with the LV cell.

There are remarkable parallels between the LV cells and other neurones that process bilateral sensory input, most notably the omega cells of crickets². Omega cells receive direct excitatory input from the receptors of the ipsilateral tympanum and indirect inhibitory input from the receptors of the contralateral tympanum, exactly paralleling the input pattern of the LV cells. The omega cells are thought to enhance bilateral differences in sound intensity and thereby help the cricket locate mates.

The role of the eyes (and in turn that of the LV cells) in behaviour is less certain but central integration of the input

from the 10 eyes could provide the leech with a coarse representation of its surroundings that could be important in a variety of behaviours. For example, like the mollusc *Hermissenda*¹¹, which has a single bilateral pair of simple eyes, the leech may use interneurons like the LV cells that compare light intensity on its two sides to guide phototaxis. However, with 10 rather than 2 eyes the leech visual system could be expected to do more than simply compare left and right, particularly given that some caterpillars, whose visual system comprises only 6 bilateral pairs of simple eyes (stemmata)^{12,13}, reportedly can discriminate between certain shapes¹⁴. Wilson¹⁵ notes that in addition to simple form recognition a set of simple eyes aimed in different directions is ideally suited for certain

dynamic visual processing tasks. For example, he suggests that in the locust, antagonism between the input from simple eyes (ocelli) that scan different sectors of the horizon could detect pitch and roll during flight, a suggestion that has received recent experimental support¹⁶. By analogy the eyes of the leech, linked to a network of fast directionally sensitive neurones like the LV cells, could conceivably detect instability during swimming.

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A possible morphogen controlling differentiation in *Dictyostelium*

R. R. Kay & K. A. Jermyn

Imperial Cancer Research Fund, Mill Hill Laboratories,
Burtonhole Lane, London NW7 1AD, UK

The complex morphology of a higher organism is generated partly by such developmental processes as cell movement and cohesion but also by a social interaction between cells in small areas of embryonic tissue known as morphogenetic fields. The initially similar cells within such a field organize themselves and differentiate, forming a discrete spatial pattern which is remarkably independent of field size and which can regenerate after some part is removed¹. Although it is believed that a cell signalling system must underlie this behaviour^{2,3}, the putative signals—or morphogens—have so far proved elusive. Perhaps the simplest known morphogenetic field arises within the multicellular aggregate formed by developing cells of the slime mould *Dictyostelium discoideum*. As the amorphous aggregate transforms into a cylindrical slug, a simple pattern emerges, with prestalk cells differentiating in the anterior and prespores in the posterior^{4,5}. One great difficulty in identifying any morphogen has been to predict properties that could form the basis of a bioassay. However, in *Dictyostelium* it is almost essential that the morphogens should dictate to cells their choice of differentiation pathway. We have described previously a crude factor termed DIF which stimulates the differentiation of isolated amoebae into stalk cells^{6,7}. We now show that purified DIF also inhibits spore formation and so switches cells to stalk cell formation. Thus, we believe that DIF is a morphogen which regulates the choice of differentiation pathway of cells in the *Dictyostelium* slug.

The present experiments used techniques for inducing the differentiation of isolated spore cells *in vitro*⁸ and for the purification of DIF. Starved amoebae can be induced to differentiate *in vitro* by incubation in tissue culture dishes in a buffered salt solution containing cyclic AMP. In these conditions amoebae of strain V12M2 are capable of differentiating into prespore and stalk cells but not into mature spores. However, mutants have been isolated from V12M2 which can complete spore differentiation^{6,9}. At low cell density these 'sporogenous' strains form only spores but at higher densities

some stalk cells also form⁸. Thus, the sporogenous strains are ideal for testing for agents, such as DIF, which might switch cells between stalk and spore formation.

The DIF used in these experiments was collected from developing cells across a dialysis membrane⁷. It was purified using XAD-2 resin, hexane extraction and two steps of reverse-phase HPLC (see Fig. 1 legend). The final product was purified about 10⁵-fold over the dialysate, had an apparent molecular weight of less than 500 and was active at less than 10⁻¹⁰M (R. K., B. Dhokia and K. A. J., in preparation).

The key experiment is to add DIF to sporogenous amoebae that would otherwise become spores. It is clear from Fig. 1b that when this is done DIF suppresses spore formation and redirects the cells to stalk cell formation. Note also that spore formation was similarly suppressed in sporogenous mutants derived from NC4, the most commonly used laboratory strain of *Dictyostelium* (Fig. 1a). Strain HM45 in fact forms a high percentage of stalk cells and thus is the first NC4-derived strain that we have found to make stalk cells efficiently *in vitro*⁶; DIF responsiveness is therefore not restricted only to V12M2-derived strains, as has been suggested.

Although Fig. 1 clearly shows that DIF blocks some step in spore differentiation it is not clear when this step might occur^{9,10}. However, if DIF is to control the differentiation of prestalk and prespore cells as we might hope, it should directly inhibit prespore cell formation. Prespore cells can be recognized using a specific antibody^{11,12} and Fig. 2 shows that DIF completely inhibits prespore cell differentiation when present from the time of plating (*t*₀). We have no equivalent way of recognizing single prestalk cells but we believe that DIF must stimulate prestalk formation in these conditions, as it does at high cell density in certain DIF-deficient mutants¹³.

As stated earlier, a morphogenetic field is often capable of reorganizing itself into a properly proportioned miniature when some part is removed by surgery. *Dictyostelium* shows this capacity to a remarkable degree and it has long been known that prespore isolates can regulate to reform a new prestalk region⁴. When this happens a proportion of the prespore cells redifferentiate into prestalk and eventually into stalk cells^{14,15}. To determine whether DIF is capable of inducing such a redifferentiation we disaggregated slugs after 18 h of development and incubated the separated cells *in vitro* in the presence of cyclic AMP. In these conditions, 50–60% of the cells stained as prespores, but when DIF was added most cells were redirected to stalk formation and there was a rapid loss of prespore staining (Fig. 3).

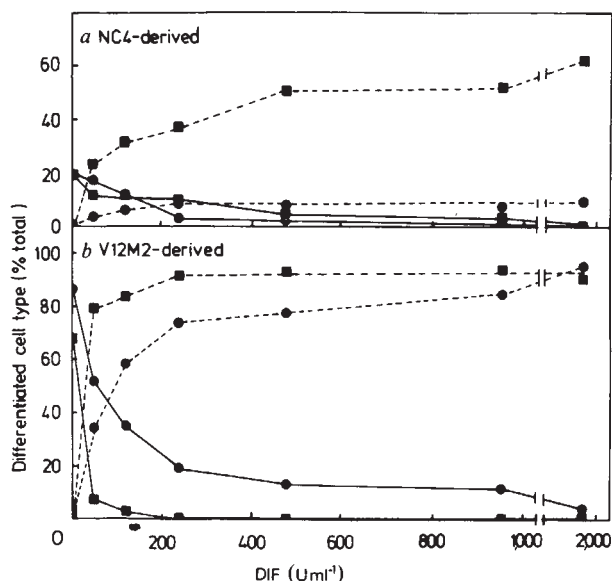


Fig. 1 DIF redirects cells of sporogenous mutants from spore to stalk cell formation. In both panels solid lines represent spore cells, and broken lines stalk cells. *a*, ●, Fr17; ■, HM45; ○, HM18; *b*, ■, HM29. Amoebae were grown on SM plates with bacteria, and development was initiated by centrifuging them free of bacteria described previously^{8,11}. The V12M2-derived sporogenous strains HM18 and HM29 have been described elsewhere^{6,8}, as has the NC4-derived strain Fr17 (ref. 19). The sporogenous strain HM45 was isolated by S. Ishida in this laboratory from the NC4-derived strain XP55 (obtained from Dr P. C. Newell)²⁰. Washed cells were plated at a density of 5×10^2 cm⁻² in 3.5-cm diameter tissue culture dishes (Sterilin) containing 0.8 ml of medium consisting of 20 mM KCl, 20 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM cyclic AMP, 15 µg ml⁻¹ tetracycline, 200 µg ml⁻¹ streptomycin, 10 mM 4-morpholinoethanesulphonic acid pH 6.2 plus 20 µg ml⁻¹ bovine serum albumin⁸. DIF dissolved in ethanol was added (up to 1 µl ml⁻¹) as indicated in the figure and the cells were allowed to differentiate for 2 days at 22°C after which time stalk, spore and undifferentiated cells were scored by phase-contrast microscopy. DIF was collected from developing cells across a dialysis membrane⁷ and bound to the nonpolar resin XAD-2 (BDH). It was eluted with ethanol, concentrated to a small volume and dispersed in water, from which the DIF was extracted into hexane. Whatman ODS columns were used for HPLC and in the first HPLC step, DIF was eluted with 70% methanol/2% acetic acid. The major peak of DIF activity (>95% of that recovered) was further purified by a second HPLC step with a gradient of 40–100% acetonitrile/5% acetic acid where it eluted as a single activity peak associated with a well resolved absorbance peak (ref. 21 and R. R. K., B. Dhokia and K. A. J., in preparation). DIF was assayed by its ability to induce stalk cell differentiation among isolated amoebae of strain V12M2 incubated with cyclic AMP as described by Brookman *et al.*¹⁶. One unit induces 1% stalk cells in the standard 2 ml assay.

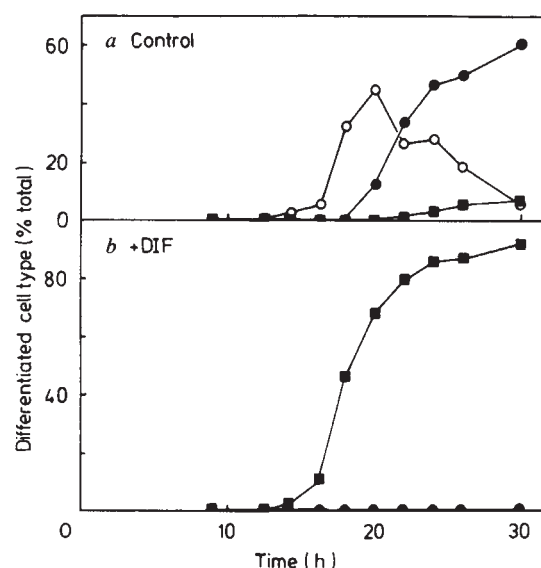


Fig. 2 DIF inhibits prespore cell differentiation. ○, Prespore cells; ●, spore cells; ■, stalk cells. Cells of strain HM18 at a density of 2.5×10^3 cm⁻² were allowed to differentiate with cyclic AMP in the same conditions as described in Fig. 1 legend. DIF was added to a concentration of 600 U ml⁻¹ in *b*. At the times indicated the incubation medium was carefully removed and the cells, which remained attached to the plastic, were fixed *in situ* with 60% followed by 100% methanol and stained with anti-spore serum (obtained from Drs D. Garrod and D. Forman)^{11,12}. Prespore, stalk and spore cells were scored using a fluorescence microscope.

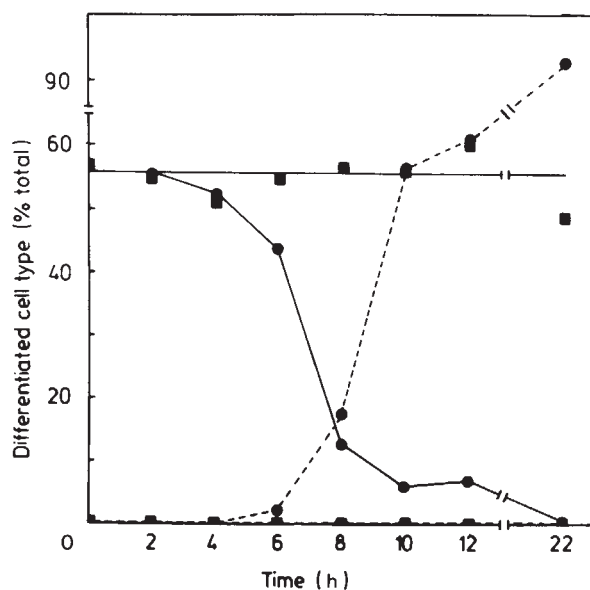


Fig. 3 DIF induces redifferentiation of prespore cells into stalk cells. Solid lines represent prespore cells, broken lines represent stalk cells. ■, No DIF; ●, +DIF. Slugs of strain V12M2 formed after 18 h of development and migrating in unidirectional light were collected, disaggregated with pronase/2,3-dimercapto-propanol¹¹ and the separated cells washed twice before being plated at a density of 3.3×10^3 cm⁻² with cyclic AMP in the same conditions as in Fig. 1. DIF was added to a concentration of 600 U ml⁻¹ as indicated. At various times cells were fixed and stained as in Fig. 2. *t*₀ in the graph is the time of plating, about 40 min after disaggregation of the slugs.

Although all the experiments described here have used highly purified DIF, there is still a possibility that the observed spore-inhibiting activity was due to a contaminant in the DIF preparation. We consider this extremely unlikely because DIF and the spore-inhibiting activity co-elute exactly on two different HPLC systems (unpublished observation) and because the putative contaminant would have to inhibit at the same very low concentration as DIF itself. Therefore, we conclude that DIF can redirect amoebae from the spore to stalk pathway of differentiation *in vitro*. We further believe that DIF also controls the pathway of differentiation of cells in normal development, because DIF is released by cells at the time prestalk cells arise¹⁶ and because mutants that are specifically DIF-deficient are blocked in development and cannot make prestalk cells but can still make prespores¹³.

We have also searched for morphogens that might oppose the actions of DIF on cell differentiation and Gross *et al.*¹⁷ have found that ammonia, which is released in substantial amounts during development¹⁸, inhibits stalk and favours spore cell formation. In addition, there is strong circumstantial evidence that DIF and ammonia have their opposing effects on cell differentiation through opposing effects on intracellular pH (ref. 17). Thus, in addition to furthering the understanding of a morphogenetic field, this system, in which purified morphogens can be used to direct cells down either of two alternative pathways for investigating the intracellular mechanisms whereby such developmental choices are made.

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Note added in proof: We have recently detected additional minor DIF activities by HPLC; only the major species (now termed DIF-1) was used for the work reported here.

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Intracellular pH and the control of cell differentiation in *Dictyostelium discoideum*

J. D. Gross, J. Bradbury, R. R. Kay & M. J. Peacey

Imperial Cancer Research Fund, Mill Hill Laboratories, Burtonhole Lane, London NW7 1AD, UK

During development in the cellular slime mould *Dictyostelium discoideum* starved amoebae aggregate to form multicellular structures that display a simple antero-posterior pattern: pre-stalk cells occupy the front 20% of the aggregate, and prespore cells occupy the remainder^{1–4}. We have attempted to elucidate the nature of the mechanism regulating the proportions of the two cell types^{1,5,6} by examining the factors that influence the pathway of differentiation of amoebae *in vitro*. Amoebae of *D. discoideum* strain V12 M2 form stalk cells efficiently in appropriate conditions and 'sporogenous' derivatives produce spores as well as stalk cells^{7,8}. Mature spores are formed in a medium containing only cyclic AMP and salts⁹, whereas formation of stalk cells requires, in addition, a low molecular weight hydrophobic factor (DIF)^{7,10}. Recent observations have led us to propose that DIF is a morphogen responsible for activating stalk cell differentiation^{5,11–13}. Here we present evidence that ammonia is a second morphogen, that acts antagonistically to DIF, and that the choice of differentiation pathway is mediated by intracellular pH.

To test for the presence of an inhibitor of stalk cell differentiation, aliquots of conditioned medium freed of DIF were added to amoebae of strain V12 M2, or of its sporogenous derivative,

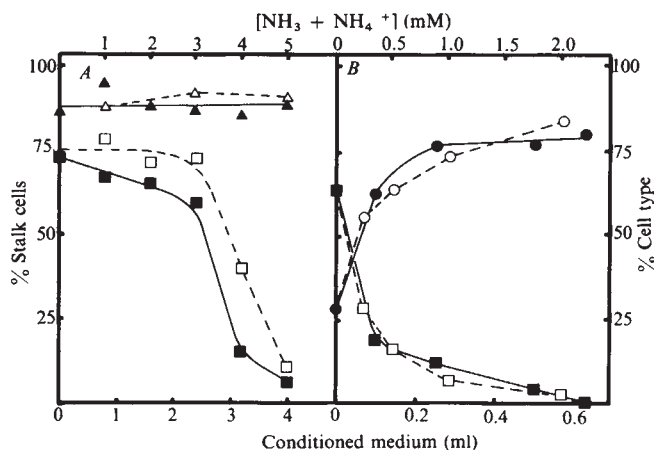


Fig. 1 The effects of conditioned medium and NH_4Cl on differentiation of amoebae of strain V12 M2 (A) and a sporogenous derivative HM18 (B). Solid symbols refer to conditioned medium, open circles to pure NH_4Cl solution. Triangles, % stalk, pH 6.2; squares, % stalk, pH 7.5; circles, % spore, pH 7.5. **Methods:** Amoebae were grown and collected as described previously³⁹ and plated in 4 ml standard stalk salts medium (SSM) buffered with 10 mM Tris (pH 7.5) or methane ethane sulphate (MES) (pH 6.2), at a density of 10^5 cells per cm^2 in 5 cm Nunc tissue culture dishes. SSM contains 10 mM KCl, 2 mM NaCl, 1 mM CaCl_2 , 200 $\mu\text{g ml}^{-1}$ streptomycin, 15 $\mu\text{g ml}^{-1}$ tetracycline and 5 mM cyclic AMP. Where indicated, aliquots of conditioned medium or NH_4Cl were added to the cells which were scored by phase-contrast microscopy for % stalk (A) or % stalk and spore (B) after incubation for 48 h at 22 °C. Conditioned medium was made by shaking 3×10^7 amoebae per ml at 80 r.p.m. in 5% Bonners salts³⁹ for 24 h. Cells and debris were removed by centrifugation at 10,000 r.p.m. and the supernatant was incubated with Amberlite XAD-2 (ref. 40) for 2 h to absorb DIF. Similar results were obtained with conditioned medium prepared by a different method¹⁰. Ammonia was determined with Nessler's reagent. Different conditioned media were used in A and B. Neither conditioned medium nor NH_4Cl had any effect on the differentiation of HM18 amoebae at pH 6.2 (data not shown).

HM18, and the amoebae incubated at densities where they produce sufficient DIF to form substantial numbers of stalk cells. No inhibition of stalk cell formation was detected when the cells were incubated at pH 6.2 (solid triangles, Fig. 1A), but stalk formation was strongly inhibited at pH 7.5 (solid squares, Fig. 1A, B), and in HM18 spore formation (Fig. 1B, solid circles) was stimulated concomitantly with inhibition of stalk formation. One interpretation of the pH dependence of inhibition is that the inhibitory substance is the neutral form of a weak base. Ammonia is an obvious candidate because large amounts are released by developing amoebae¹⁴. Moreover, Schindler and Sussman¹⁵ have demonstrated that ammonia controls the transformation of cell aggregates into migratory slugs, and themselves proposed that ammonia acts as inhibitor of stalk cell differentiation¹⁶. The conditioned media used in the experiments detailed in Fig. 1 were found to contain levels of ammonia consistent with previous measurements^{15,17,18}. Replacement of the conditioned medium with pure ammonium chloride (or other ammonium salts) showed that the inhibitory effect could be quantitatively accounted for by the ammonia content (see dotted lines in Fig. 1A, B). Addition of equivalent amounts of KCl and NaCl had no effect. A brief report of the results with ammonium salts has been presented previously⁵. In addition, inhibitory activity co-distilled with ammonia at pH 5.0 and pH 10, and was destroyed by incubation with a glutamate dehydrogenase enzyme mix in which ammonia is a substrate¹⁵ (data not shown). Ammonia was also found to inhibit entry of cells into the prestalk pathway rather than, for example, blocking some step in stalk maturation¹⁹; its effect is antagonized by DIF (M.J.P. and J.D.G., unpublished observation).

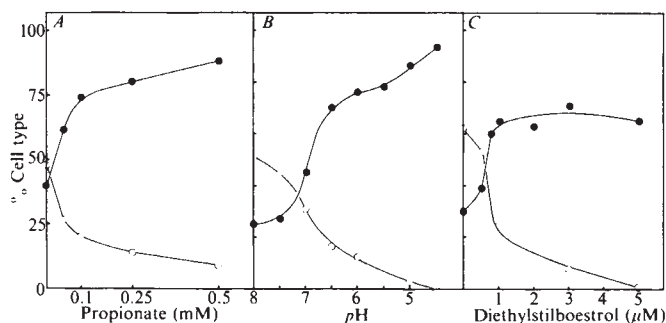


Fig. 2 The effect of propionate (A), extracellular pH (B), and diethylstilboestrol (C) on stalk and spore formation. HM18 amoebae were grown and collected as described in the legend to Fig. 1. A, Amoebae incubated at 2×10^4 per cm^2 in spore induction medium⁹ containing 2 mM cyclic AMP and buffered at pH 5.0 with 10 mM MES. B, SSM medium adjusted to the required pH with 10 mM McIlvaine buffer⁴¹. Amoebae at 10^5 per cm^2 . C, As in A except pH 6.2. ●, Stalk cells; ○, spores.

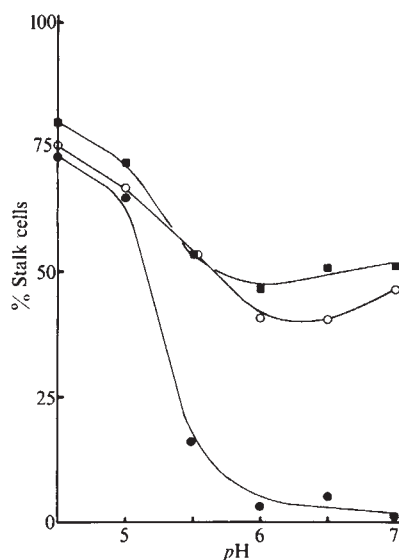


Fig. 3 The effect of pH_0 , DIF and diethylstilboestrol on stalk formation by V12 M2 amoebae at low density. Amoebae were grown and collected as described in Fig. 1 legend and plated at 5×10^3 cm^{-2} in SSM buffered with 10 mM McIlvaine buffer. ●, Control; ■, DIF (40 units ml^{-1}); ○, DES (3 μM).

There is evidence that ammonia and other weak bases influence various biological processes by diffusing into the cell where they become protonated and cause a rise of intracellular pH_i^{20-24} (henceforth abbreviated to pH_i). It appeared, therefore, that the choice of pathway of differentiation in *Dictyostelium* might depend on pH_i , high pH_i (induced by ammonia) inhibiting stalk and favouring spore formation and low pH_i (brought about in some way by DIF) giving the reverse effect. The following observations support this view: (1) other weak bases such as trimethylamine, methylamine, imidazole and procaine mimic the effect of ammonia, and weak acids have the opposite effect. Thus exposure of HM18 amoebae to increasing concentrations of propionate at pH 5.0 caused progressive diversion from the spore to the stalk pathway (Fig. 2A) and acetate, butyrate and isobutyrate were also effective. As expected, the potency of the weak acids decreased as the pH of the medium was raised. (2) Systematic reduction of pH_0 (extracellular pH) shifts the pattern

of cell differentiation towards stalk formation (see Fig. 2B). This effect of pH_0 has been confirmed with HM29, a different sporogenous derivative of strain V12, and also with sporogenous derivatives of strain NC4 (B. Dhokia, personal communication).

Our work on the purification of DIF indicates that it is effective at very low concentrations, and hence does not simply behave as a weak acid. How then might it act to decrease pH_i ? An attractive possibility is that it inhibits the export of protons from the cell. In different systems proton export occurs either by electroneutral exchange with other monovalent cations²⁵⁻²⁷ or by an energy-dependent electrogenic process^{28,29}. Plasma membrane ATP-dependent proton pumps have been demonstrated in fungi^{30,31} (including *Physarum*, an acellular slime mould³⁰), as well as in algae and higher plants²⁸, and the synthetic oestrogen, diethylstilboestrol, has been shown to be a potent inhibitor of these plasma-membrane H^+ -ATPases³¹⁻³³. We therefore tested the effect of diethylstilboestrol on stalk and spore cell differentiation and found that very low concentrations were able to divert cells from the spore to the stalk pathway (Fig. 2C). Moreover, as shown in Fig. 3, diethylstilboestrol, unlike many substances that we had tested previously, mimics the action of DIF in inducing stalk cell formation when added to isolated cells of strain V12 M2. A second proton pump inhibitor, the naturally-occurring oestrogen, zearalenone³⁴, also mimics the actions of DIF (data not shown). The results shown in Fig. 3 demonstrate in addition that incubation of isolated amoebae at low pH_0 is by itself sufficient to induce stalk cell formation. These results thus support the idea that DIF acts to lower intracellular pH by inhibiting an ATP-dependent proton pump.

There is increasing reason to believe that pH_i has an important role in the control of cell proliferation in higher animals^{24,35-38}. The results reported here constitute the first evidence that pH_i can determine the choice between alternative pathways of cell differentiation.

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Immunospecific inhibition of nerve conduction by T lymphocytes reactive to basic protein of myelin

Yosef Yarom*, Yaakov Naparstek†‡, Varda Lev-Ram†, Joseph Holoshitz†§, Avraham Ben-Nun† & Irun R. Cohen†

* Department of Neurobiology, Institute of Life Sciences, Hebrew University, Jerusalem, Israel

† Department of Cell Biology, The Weizmann Institute of Science, PO Box 26, Rehovot 76100, Israel

‡ Department of Medicine A, Hadassah University Hospital, Jerusalem, Israel

§ Department of Medicine B, Meir General Hospital, Kfar-Saba, Israel

Experimental autoimmune encephalomyelitis (EAE) induced by immunization to the basic protein of central nervous system myelin (BP) is a paralytic disease in which T lymphocytes attack the individual's own central nervous system¹. As the target is in white matter, EAE has been considered an experimental model of some aspects of human disease such as multiple sclerosis. To investigate whether autoimmune T lymphocytes could produce paralysis, we studied the effects on the electrophysiology of isolated nerves produced by T-lymphocyte lines reactive specifically to BP or other antigens. We now report that propagation of action potentials evoked by electrical stimulation was blocked by incubating optic nerves with specific anti-BP T cells. This blockade could be reversed for up to two hours by removing the anti-BP line cells from the optic nerve. The anti-BP line cells had no effect on conduction along allogeneic optic nerves or syngeneic peripheral nerves. This indicates that disruption of the function of myelin in neuroimmunological disease may result from an immunologically specific interaction between autoimmune T lymphocytes and myelin antigens.

We have succeeded in isolating and growing as long-term cell lines T lymphocytes reactive to BP or to other antigens²⁻⁴. Anti-BP T cells were found to mediate EAE within several days of intravenous inoculation into naive recipient rats. The rats developed paralysis and showed the same perivascular inflammatory cell infiltration as found in EAE induced by active immunization to BP in complete Freund's adjuvant. Moreover, anti-BP line cells, attenuated by treatment with mitomycin C or irradiation, could be used to vaccinate rats against subsequent induction of active EAE by immunization^{5,6}.

To investigate whether anti-BP T lymphocytes could disrupt nerve conduction, we constructed a chamber in which it was possible to observe conduction in an isolated nerve in the presence of test lymphocytes (Fig. 1). Each end of a section of nerve was aspirated into the tip of a suction electrode that could be used either for stimulation or recording. Propagation of action potentials was studied in a single set of axons before, during and after incubation of the nerve with T cells.

We used T cells from Lewis rat-derived lines directed against BP or against the purified protein derivative (PPD) of *Mycobacterium tuberculosis*²⁻⁴, and assayed their effects on optic nerves containing BP of central myelin or on sciatic nerves containing an immunologically distinct peripheral myelin⁷. Nerves were obtained from Lewis rats syngeneic to the Lewis line cells, or from BN strain rats whose major histocompatibility complex (MHC) genes differ from those of Lewis rats⁸. Before use, it was necessary to activate each population of T-lymphocyte line cells by incubation for 72 h with its specific antigen, either BP or PPD, in the presence of irradiated (1,500 R) thymus cells from normal syngeneic rats as antigen-presenting accessory

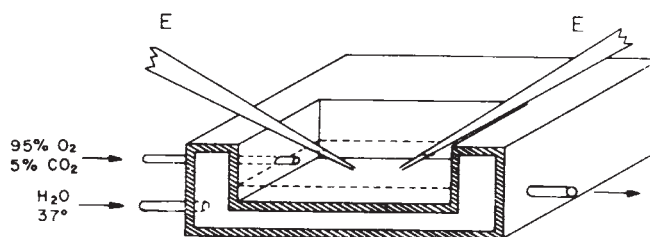


Fig. 1 Chamber for study of physiology of an isolated nerve. Segments of optic or sciatic nerves of 5 mm were obtained from decapitated rats and suspended in the chamber by aspirating each end into a suction electrode (E). The chamber was bathed in H₂O kept at a temperature of 37°C and exposed to a constant flow of 95% O₂ and 5% CO₂. The nerve was immersed just under the surface of the Eagle's tissue culture medium. The portions of nerve within the suction electrodes were not exposed to the medium in the bath into which the lymphocytes were introduced during testing. Hence stimulating and recording conditions remained the same during the experiments. Cells were added to the medium at a concentration of 2×10^5 per ml. Three days before testing, the cells were activated by incubation with their specific antigen, either BP of central myelin or PPD, in the presence of irradiated (1,500 R) syngeneic thymocytes as accessory cells²⁻⁴.

cells^{2-4,9}. The large majority of irradiated accessory cells had died after 72 h and about 80–90% of the remaining cells were T lymphoblasts. The cells were washed by centrifugation and resuspended for testing in fresh medium that did not contain either BP or PPD antigens.

Blockade of action potentials by incubation of Lewis optic nerve with Lewis anti-BP T cells is illustrated in Fig. 2. Figure 2a shows a stepwise increase in the amplitude of stimuli; the compound action potential in optic nerve generated by stimuli of increasing voltage intensities before incubation with line cells. The two superimposed traces shown in Fig. 2b were obtained before (upper) and after (lower) incubation of the nerve with line cells for 40 min. The marked decrease in the amplitude of the induced action potential suggests a reduction in the number of functioning axons is caused by incubation with anti-BP T cells. This blockade could not be explained by an increase in the firing threshold because it was not reversed by increasing the intensity of the stimulus (Fig. 2c). Figure 2d–f show that the blockade produced by incubation with anti-BP T cells was reversible. The control response of the optic nerve to various stimulus intensities before incubation is illustrated in Fig. 2d, after incubation in Fig. 2e, and after removal of the anti-BP line cells in Fig. 2f. The blockade was not reversible after incubation of optic nerves with anti-BP T cells for longer than 2 h.

The immunological specificity of the blockade is shown in Fig. 3. It can be seen that conduction was affected by incubating Lewis optic nerves with anti-BP T cells (Fig. 3a) but not with anti-PPD line cells (Fig. 3b). Conduction was not affected in BN optic nerves incubated with Lewis anti-BP cells (Fig. 3c), nor in Lewis sciatic nerves incubated with Lewis anti-BP cells (Fig. 3d). Blockade was not produced by cell-free culture medium collected from anti-BP or anti-PPD cell lines that had been incubated with their specific antigen in the presence of irradiated antigen-presenting cells (not shown).

The immunospecificity of the interaction suggests that blockade of the nerve impulse required the recognition by the anti-BP T lymphocytes of the BP target antigen together with strain-

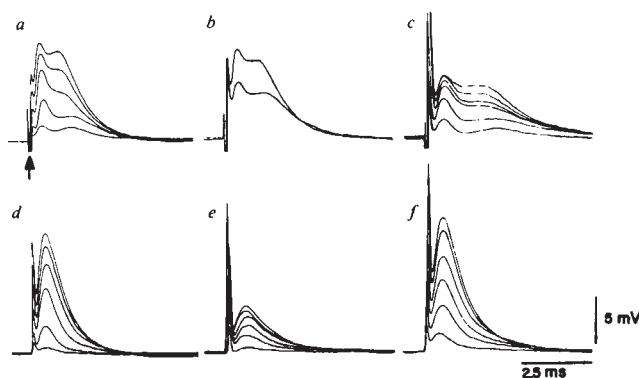


Fig. 2 Blockade of action potential along optic nerve by anti-BP line cells. Anti-BP T-cell lines were raised, maintained, and activated by incubation with BP in the presence of irradiated (1,500 R) syngeneic thymus cells as described⁴. The line cells (2×10^5 per ml) were incubated with Lewis optic nerves for 40 min and the optic nerve was stimulated by 0.05 ms pulses of various amplitudes produced by an isolated stimulator (Devices, MK IV, London). The compound action potential was recorded using a differential amplifier connected to a digital oscilloscope (Nicolat) and an x-y recorder. The arrow indicates a stimulus artefact. *a*, Before incubation with the line cells using stimuli of 0.5, 1, 2, 3 and 4 V. *b*, Recording before and after 40 min of incubation with line cells using a stimulus of 3 V. *c*, Multiple stimuli made after incubation with anti-BP line cells at intensities 1, 2, 3, 4, 5 and 6 V. *d*, Recording from a different optic nerve before incubation with anti-BP line cells using stimuli of 0.25, 0.5, 1, 1.5, 2 and 2.5 V. *e*, The response after 60 min of incubation using stimuli of 1, 2, 3, 4, 5 and 6 V, and *f*, after the line cells had been washed out of the chamber using 0.5, 1, 1.5, 2, 2.5 and 3 V.

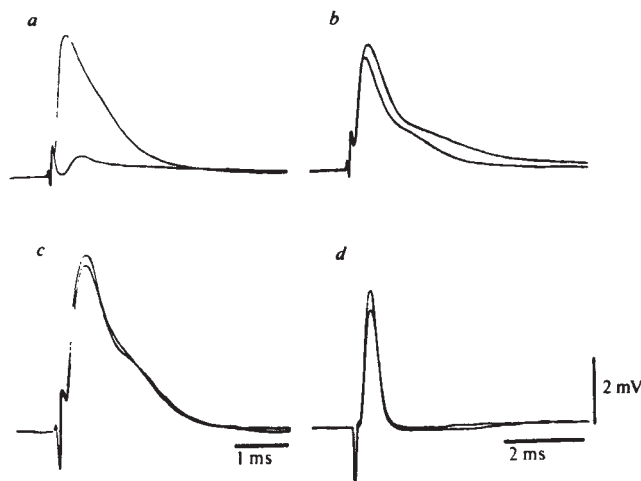


Fig. 3 Immunological specificity of blockade of nerve conduction. The preparations were as described in the legend to Figs 1 and 2. The upper curve was recorded before incubation and the lower curve after incubation with T cells. *a*, The effect of incubating a Lewis optic nerve with syngeneic anti-BP line cells. *b*, The effect of Lewis anti-PPD line cells on a Lewis optic nerve. *c*, The effect of Lewis anti-BP line cells on an optic nerve originating from an allogeneic BN rat. *d*, The effect of anti-BP line cells on syngeneic sciatic nerve.

specific self markers. We found that anti-BP lymphocytes proliferated *in vitro* when presented BP by accessory cells syngeneic at the MHC but did not respond to BP presented by accessory cells with MHC genes of BN origin⁴. Associative recognition of MHC together with target antigen appears to be a general property of effector T lymphocytes¹⁰ and appears to be occurring here.

At present we do not know whether the line cells recognize BP together with self-MHC on antigen presenting cells or on the optic nerve itself. Before exposure to the nerves, the anti-BP line had been activated by incubation with BP in the presence of syngeneic accessory cells, yet the BN optic nerve and the Lewis sciatic nerve were not affected. Thus, dual recognition of BP and self-MHC mediated by the accessory cells before incubation with the nerve was not sufficient for the T-cell line to interrupt the nerve impulse. This argues that the inhibitory effect may require recognition of both BP and MHC gene products on the nerve itself. MHC gene products have not been demonstrated on optic nerves, but it is possible that they were expressed there because murine brain cells have been shown to express Ia antigens¹¹ and HLA-DR antigens appear to be present on human glial cells¹². The specificity of inhibition of nerve conduction also argues against the possibility that macrophage-like accessory cells were the mediators of the inhibition of nerve conduction. Such macrophage-like cells were present in the control cultures of the activated anti-PPD T cells incubated with optic nerve, and in the cultures of activated anti-BP T cells incubated with either syngeneic peripheral nerve or allogeneic optic nerve. Yet no inhibition of conduction was seen.

Previous studies have claimed that sera obtained from patients with multiple sclerosis or animals with EAE can affect the electrical activity of cultured nervous tissues^{13,14}. Evidence that nerve conduction can be blocked by antibodies to components of myelin was obtained in studies using antiserum to galactocerebroside¹⁵. However, it is highly unlikely that our lines of immunoglobulin-negative T lymphocytes⁴ could have secreted antibodies that blocked nerve conduction. Demyelina-

tion of nerves produced by sensitized lymphocytes has been observed in tissue culture¹⁶. The present system extends these investigations as we have shown an immunologically specific and reversible blockade of nerve conduction. Two general processes could be responsible for blockade of conduction in myelinated nerve fibres; changes in the current generating mechanisms due to changes in channel properties or ion gradients¹⁷, and alteration in fibre geometry due to disruption of myelin¹⁸. Although the mechanism has yet to be explored, it is likely that conduction block was initiated by changes in myelin, the site of the BP target antigen.

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Differentiation *in vitro* of Lyt 2⁺ thymocytes from embryonic Lyt 2⁻ precursors

Rhodri Ceredig, Rafick P. Sekaly
& H. Robson MacDonald

Ludwig Institute for Cancer Research, Lausanne Branch,
1066 Epalinges, Switzerland

In mice, the thymus is regarded as being the primary anatomical site for the generation of immunologically competent T lymphocytes¹. Such cells comprise approximately 20% of the cells in the thymus and share with T lymphocytes from peripheral lymphoid tissues certain phenotypic properties defined by anti-Lyt antibodies^{2,3}. Thus, most immunocompetent T cells are either Lyt 1⁺2⁺ or Lyt 1⁺2⁻ with the former cells being restricted to recognizing antigen in association with class I (H-2K, D) major histocompatibility complex (MHC) products and the latter to class II (H-2I) MHC products⁴. Although evidence suggests that Lyt 1⁺2⁺ cells are generated from Lyt 1⁺2⁻ precursor^{5,6} the independent development of two separate Lyt-defined lineages of thymocytes could not be ruled out^{5,7}. Here, the acquisition of Lyt 2 antigen by Lyt 2⁻ cells from late embryonic and early postnatal thymuses is directly demonstrated. Furthermore, by combining cell cycle and Lyt phenotype analysis on a flow microfluorometer, the role of cell division in this differentiation process has been investigated.

The phenotypic and functional dichotomy of T cells and the concomitant expression of class I and II MHC molecules by non-lymphoid thymic components⁸ has been interpreted as indicating a site-specific maturation and consequent MHC restriction of Lyt-differentiated thymocytes⁵. Originally, it was postulated that both Lyt-defined mature thymocytes arose from an immature Lyt 1⁺2⁺ precursor cell⁹. During mouse embryonic development, however, Lyt 2 antigen first appears on a subpopulation of cells comprising approximately 20% of thymocytes at the 16th day of gestation^{5,10} but from 16 to 19 days, the embryonic thymus grows exponentially and the proportion of Lyt 2⁺ cells increases to 80%. These results, together with studies showing the appearance of Lyt 1⁺2⁺ cells in organ-cultured embryonic thymuses^{6,10} suggested that Lyt 1⁺2⁺ cells were generated from a Lyt 1⁺2⁻ precursor.

To test this possibility directly, Lyt 2⁻ cells, obtained by monoclonal anti-Lyt 2 antibody and complement treatment of thymocytes from fetal mice of known gestational age, were cultured at 37°C *in vitro* at high cell density in the wells of microtitre plates. These Lyt 2⁻ fetal thymocytes stained very weakly with a monoclonal anti-Lyt 1 antibody compared with Lyt 2⁻ cells from the adult thymus. After 20 h *in vitro*, the surviving cells were stained by indirect immunofluorescence with a monoclonal anti-Lyt 2 antibody and analysed on a fluorescence-activated cell sorter (FACS II) flow microfluorometer. Figure 1 shows the results of a typical experiment. Clearly, after overnight culture at 37°C a subpopulation (44%) of the original Lyt 2⁻ fetal thymocytes now expresses Lyt 2 antigen.

In the above experiments, the viable cell recovery from microtitre wells was approximately 50%. Even though contamination of the starting Lyt 2⁻ population with Lyt 2⁺ cells was very low ($2 \pm 1.5\%$, $x \pm s.d.$, $n = 10$) it was nevertheless important to ensure that the Lyt 2⁺ cells detected after 20 h were not the progeny of this small number of contaminating cells. This was done by using thymocytes from strains of mice expressing different Lyt 2 alleles¹¹. Thus Lyt 2⁻ thymocytes from newborn DBA/2 (H-2^d, Lyt 2.1) mice were mixed with 10% BALB/c (H-2^d, Lyt 2.2) age-matched untreated thymocytes and the resulting cell mixture cultured for 20 h and stained with either a non-polymorphic (anti-Lyt 2) or polymorphic (anti-Lyt 2.2)

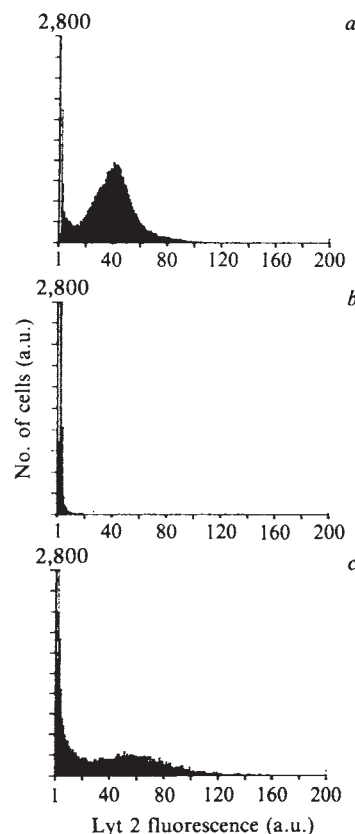


Fig. 1 The generation of Lyt 2⁺ cells following *in vitro* culture of Lyt 2⁻ thymocytes from 19-day-old mouse embryos. Each histogram, normalized to peak height, shows the Lyt 2 fluorescence profile obtained using a FACS II flow microfluorometer of 40,000 viable cells (gated by forward light scatter) from: *a*, 19-day CBA/J mouse embryos; *b*, cells from *a* treated with monoclonal anti-Lyt 2 antibody plus complement; *c*, cells from *b* cultured for 20 h *in vitro*. The date of finding a vaginal plug was taken as day 0 of gestation. Cells were stained by indirect immunofluorescence using a monoclonal rat IgG anti-Lyt 2 antibody (53-6.7), given by Dr J. Ledbetter, followed by fluoresceinated rabbit anti-rat immunoglobulin (Nordic). Immunofluorescent staining was at 4°C and flow microfluorometric analysis was carried out as previously described³. Control samples were stained with the rabbit anti-rat immunoglobulin second-step reagent alone. Lyt 2⁻ embryonic thymocytes were obtained by treating 2×10^8 cells with a 1:10 dilution of monoclonal rat IgM anti-Lyt 2 antibody (3.168.8.1), a gift of Dr F. Fitch, and a 1:20 dilution of rabbit complement (Cedarlane) in a one-step cytotoxicity procedure as described previously³. Viable cells, usually <10% of the starting population, were then purified by centrifugation over Hypaque-Ficoll followed by three washes in Dulbecco's modified Eagle's medium (DMEM). An aliquot (10^6) of cells was then stained as above (*b*) and the remaining cells cultured in flat-bottomed microtitre plates (Falcon), each well initially containing 10^6 cells in a total volume of 0.2 ml. The culture medium used was DMEM supplemented with additional amino acids¹⁷, 10% (v/v) fetal calf serum and freshly added glutamine (216 mg l^{-1}), 10 mM HEPES and $5 \times 10^{-5} \text{ M}$ 2-mercaptoethanol as previously described³. After 18–20 h in culture at 37°C in a humidified atmosphere containing 5% CO₂ in air, cells in the microtitre wells were collected, the viable cell recovery determined by Trypan blue exclusion and stained as above (*c*). Compared with the 0.5% positive cells in the control sample, *a* contained 87%, *b* 0.9% and *c* 44% Lyt 2⁺ cells.

monoclonal antibody. The results (Table 1) show that the proportion of Lyt 2.2⁺ (contaminating) cells in the mixture decreased from 10 to 2% following *in vitro* culture. Reciprocal experiments in which Lyt 2⁻ BALB/c neonatal thymocytes were contaminated with age-matched DBA/2 thymocytes and cultured for 20 h, gave a similar result to that described above.

Further evidence that the Lyt 2⁺ cells detected at 20 h were not derived via proliferation of contaminating Lyt 2⁺ cells was

Table 1 $\text{Lyt } 2^+$ thymocytes arise from $\text{Lyt } 2^-$ precursor cells

	% Positive cells	
	$\text{Lyt } 2$	$\text{Lyt } 2.2$
Before culture		
DBA/2	82	0
BALB/c	79	82
DBA/2 (90%) + BALB/c (10%)	79	10
$\text{Lyt } 2^-$ DBA/2	2	n.d.
After 20-h culture		
$\text{Lyt } 2^-$ DBA/2 alone	26	0
$\text{Lyt } 2^-$ DBA/2 (90%) + BALB/c (10%)	25	2

Cells were stained by indirect immunofluorescence either with monoclonal rat IgG antibody directed against nonpolymorphic determinants of the $\text{Lyt } 2$ molecule (53-6.7) or with a mouse IgM antibody (HO-2.2), a gift of Dr P. Gottlieb, directed against allelic determinants on the $\text{Lyt } 2.2$ molecule¹⁶. % Positive cells refers to the proportion of cells staining above control samples stained with the appropriate fluoresceinated second-step reagents alone.

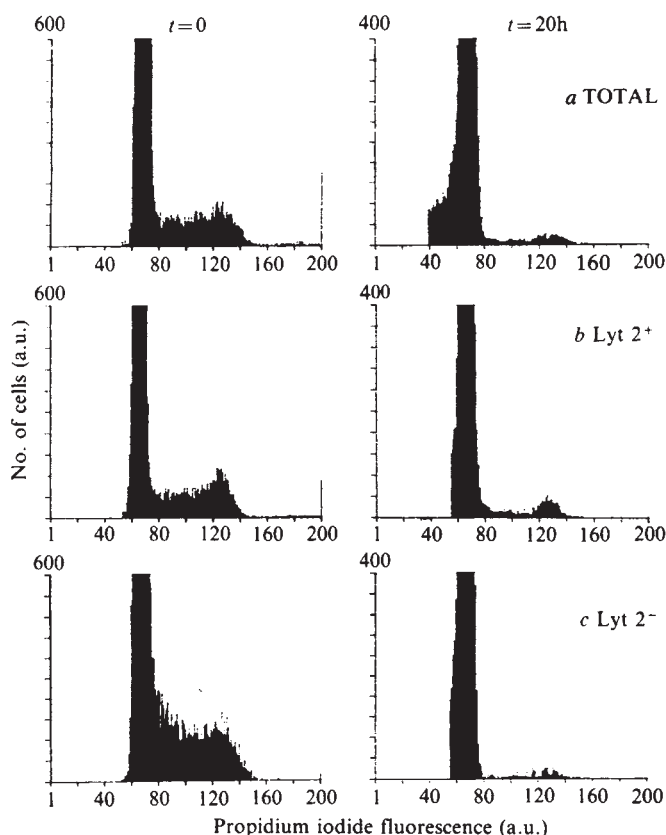


Fig. 2 Cell cycle analysis of 19-day embryonic mouse thymocytes before ($t=0$) and after 20-h culture. Each histogram, normalized to peak height, shows the propidium iodide fluorescence profile of 3×10^4 total (a), $\text{Lyt } 2^+$ (b) and $\text{Lyt } 2^-$ (c) embryonic thymocytes (left three panels) and of $\text{Lyt } 2^-$ cells from these cultured for 20 h *in vitro* (right panels). In each case, cells were stained by indirect immunofluorescence with a monoclonal anti- $\text{Lyt } 2$ antibody as described in Fig. 1 legend. Labelled cells were then passed through a FACS II and either collected unsorted (a, total) or sorted into $\text{Lyt } 2^+$ or $\text{Lyt } 2^-$ subpopulations. The sorted viable cells were then stained with the DNA-binding dye propidium iodide in the presence of 0.05% Nonidet P-40 (NP40) and analysed for their DNA content as described previously¹⁵. The major peak of cells between channels 60–80 represents cells in G_1 , the peak at 120–140 represents cells in $G_2 + M$, and cells between 80 and 120 are at the S phase of the cell cycle. The proportion of cells in S, $G_2 + M$ phases of the cell cycle ($>$ channel 80) was 14%, 13% and 25% for total, $\text{Lyt } 2^+$ and $\text{Lyt } 2^-$ cells, respectively, at $t=0$ and 3%, 3% and 1% at $t=20$ h.

provided by analysing the DNA content of the cultured cells. In these experiments, uncultured day 19 fetal thymocytes, or $\text{Lyt } 2^-$ day 19 fetal cells cultured for 20 h, were stained with monoclonal anti- $\text{Lyt } 2$ antibody and sorted into $\text{Lyt } 2^+$ and $\text{Lyt } 2^-$ cells on the FACS II. Then, the sorted cells were fixed, stained with propidium iodide and reanalysed for their DNA content. As shown in Fig. 2, $\text{Lyt } 2^-$ cells in the day 19 fetal thymus contained 25% cells in the S, G_2 and M phases of the cell cycle but by 20 h, this value had fallen to 1%. The reduced proliferation at 20 h was seen in both $\text{Lyt } 2^-$ and $\text{Lyt } 2^+$ cells. This rapid reduction in cellular proliferation of thymocytes in culture was expected from previous studies which showed the rapid death of most adult thymocytes when maintained in short-term liquid culture¹².

Table 2 summarizes experiments in which $\text{Lyt } 2^-$ thymocytes from mice of different gestational ages and genetic backgrounds were cultured for 18–20 h *in vitro* and stained with monoclonal

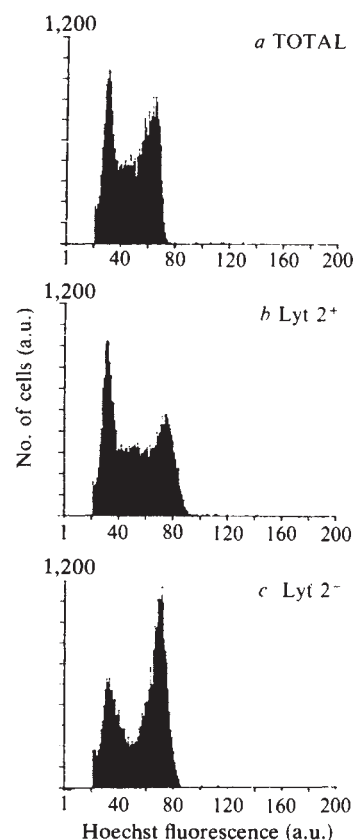


Fig. 3 Analysis of Hoechst DNA fluorescence of $\text{Lyt } 2^-$ embryonic thymocytes cultured in the presence of BrdUrd. $\text{Lyt } 2^-$ 19-day embryonic mouse thymocytes were cultured for 20 h as described in Fig. 1 legend in the presence of $2 \mu\text{g ml}^{-1}$ BrdUrd and an equimolar concentration ($1.6 \mu\text{g ml}^{-1}$) of deoxycytidine. The latter drug prevents the inhibition of ribonucleotide reductase and consequent cytotoxicity of BrdUrd¹⁸. The cultured $\text{Lyt } 2^-$ cells were collected, stained by indirect immunofluorescence with monoclonal anti- $\text{Lyt } 2$ antibody, passed through the FACS II and collected unsorted (a, total) or sorted into $\text{Lyt } 2^+$ (b) or $\text{Lyt } 2^-$ (c) subpopulations. Then the viable sorted cells were stained with the DNA-binding dye Hoechst 33342 ($1 \mu\text{g ml}^{-1}$) in the presence of 0.05% NP40 and analysed for their fluorescence intensity. Freshly removed adult mouse thymocytes, stained as above, served as a control for the G_1 peak. At $t=0$, the Hoechst staining of the $\text{Lyt } 2^-$ cells was identical to the propidium iodide distribution shown in Fig. 2c (left panel). The peak of cells at channels 60–80 in this figure represents cells remaining in the G_1 phase of the cell cycle. Cells in the peak at channels 30–40 (G_1'), having 50% the fluorescence of G_1 cells, were in G_1 at the time of BrdUrd addition and subsequently passed through mitosis. S' cells present between the G_1' and G_1 peaks (channels 40–60) were at differing stages of S at the time of BrdUrd addition. In this experiment, 64% of $\text{Lyt } 2^+$ and 37% $\text{Lyt } 2^-$ cells had quenched Hoechst fluorescence ($<$ channel 60).

anti-Lyt 2 antibody. The viable cell recovery varied between experiments as did the proportion of cells subsequently expressing Lyt 2. However, the proportion of Lyt 2⁺ cells was greatest when late fetal or early neonatal Lyt 2⁻ cells were cultured. The proportion of Lyt 2⁺ cells following culture of Lyt 2⁻ adult thymocytes was low (6%) and the intensity of staining was weak.

Several models relating the acquisition of differentiated function to the cell cycle have been proposed¹³. To study the possible role of cell cycle phase on the acquisition of Lyt 2 by Lyt 2⁻ cells, a recently described DNA staining technique was used. Hoechst 33342 is an adenine/thymidine-specific vital stain that may be used to quantify cellular DNA content. An important additional property of this dye is that if proliferating cells are cultured in the presence of the thymidine analogue bromodeoxyuridine (BrdUrd) and subsequently stained with Hoechst, the incorporated BrdUrd will quench or reduce quantitatively the fluorescence of the Hoechst dye^{14,15}. Thus, cells cultured with BrdUrd will not increase their Hoechst fluorescence during S phase and will exhibit quenched fluorescence after passing through mitosis.

When Lyt 2⁻ embryonic thymus cells were cultured in the presence of BrdUrd, a similar proportion (58%) of cells became Lyt 2⁺ as in control cultures (57%). However, in the presence of BrdUrd a proportion of cells with quenched Hoechst fluorescence was generated *in vitro* (Fig. 3). Further analysis using cell sorting showed that both Lyt 2⁺ and Lyt 2⁻ subpopulations contained cells with quenched fluorescence; however, the percentage of quenched cells was significantly greater in the Lyt 2⁺ than in the Lyt 2⁻ cells (64 versus 37%). In two other experiments, the relative proportion of quenched Lyt 2⁺ and Lyt 2⁻ cells was 63% and 59% versus 33% and 33%, respectively.

Assuming no selective loss of cells, the ratio of quenched cells in the two subpopulations demonstrates that the Lyt 2⁻ cells which did divide during the 20-h culture period gave rise to twice as many Lyt 2⁺ as Lyt 2⁻ progeny. However, it is not possible to distinguish between asymmetrical division or the acquisition of Lyt 2 antigen before mitosis as an explanation for the preponderance of Lyt 2⁺ cells with quenched DNA fluorescence. As proliferation in this *in vitro* system ceases at 24 h, it is also unclear whether Lyt 2⁻ cells with quenched Hoechst fluorescence are derived from a cell at a stage of differentiation before the transition to Lyt 2⁺, or a subpopulation of Lyt 2⁻ cells destined to remain Lyt 2⁻.

In summary, these results show unequivocally that Lyt 2⁺ cells in the embryonic thymus can arise by the division of Lyt 2⁻ precursors. Based on their weak expression of Lyt 1 and lack of specific cytolytic T lymphocyte precursor activity (refs 19,

20 and unpublished data), it would appear that these Lyt 2⁺ cells correspond to immature, or cortical, thymocytes. Finally, the combination of DNA and cell surface phenotype analysis by flow microfluorometry, as described here, should be applicable to the study of lineage interrelationships in other differentiating systems.

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Acetylcholine activation of single muscarinic K⁺ channels in isolated pacemaker cells of the mammalian heart

B. Sakmann*, A. Noma† & W. Trautwein‡

* Max-Planck-Institut für biophysikalische Chemie, 34 Göttingen, FRG

† National Institute for Physiological Sciences, 444 Okazaki, Japan

‡ II Physiologisches Institut, Universität des Saarlandes, Homburg, FRG

Acetylcholine (ACh) released on vagal stimulation¹ reduces the heart rate by increasing K⁺ conductance of pacemaker cells in the sinoatrial (S-A) node^{2,3}. Fluctuation analysis of ACh-activated currents in pacemaker tissue showed this to be due to opening of a separate class of K⁺ channels gated by muscarinic ACh receptors (m-AChRs)⁴. On the other hand, it has been suggested that m-AChRs may simply regulate the current flow through inward rectifying resting K⁺ channels (*g_{K1}*)^{5,6}. We report here the measurement of ACh-activated single channel K⁺ currents and of resting K⁺ channel currents in isolated cells of the atrioventricular (A-V) and S-A node of rabbit heart. The results show that the ACh-dependent K⁺ conductance increase in nodal cells is mediated by K⁺ channels which are different in their gating and conductance properties from the inward rectifying resting K⁺ channels in atrial and ventricular cells. The resting K⁺ channels in nodal cells are, however, similar to those activated by ACh.

Current recordings were made from enzymatically dispersed S-A and A-V nodal cells⁷. After 1–2 h of superfusion with normal Tyrode solution, many single cells appear rounded or oval (nodal 'cardioballs'). They have diameters of 15–30 µm and beat spontaneously. Membrane currents and membrane potentials were recorded via a glass pipette in the 'whole cell' and in the 'cell-attached patch' recording configuration⁸. Rounded cells had action potentials very similar to those recorded from clusters of beating cells⁷. Experiments were performed at 30–32 °C in bath solution of the following composition (in mM): NaCl 145, CaCl₂ 1.0, KCl 5.4, MgCl₂ 1, HEPES 5, pH 7.2. The pipette solution had a similar composition but K⁺ concentration was increased by replacing an equimolar amount of Na⁺, and ACh was present at 10⁻⁷–10⁻⁶ M. Nearly all cells

Table 2 Lyt 2 antigen appears on Lyt 2⁻ thymocytes from mice of different ages

Expt	Age	Mouse strain	Cell recovery (%)	Per cent Lyt 2 ⁺ cells	
				Before culture	After culture
(1)	18-day fetus	CBA	50	2.0	78
(2)	18-day fetus	CBA	60	4.7	57
(3)	19-day fetus	CBA	55	0.7	62
(4)	19-day fetus	CBA	60	0.9	44
(5)	19-day fetus	CBA	40	0.1	56
(6)	1-day neonate	C57BL/6	80	1.0	18
(7)	1-day neonate	DBA/2	60	4.0	31
(8)	1-day neonate	BALB/c	30	3.0	41
(9)	7-day neonate	C57BL/6	66	2.0	15
(10)	4-week adult	CBA	50	1.0	6

Summary of experiments with cultured Lyt 2⁻ mouse embryonic, neonatal and adult thymocytes. Lyt 2⁻ cells were obtained by monoclonal antibody and complement treatment of thymocytes as described in Fig. 1 legend. The viable cell recovery from pooled microtitre wells was determined by Trypan blue exclusion. Cells were stained by indirect immunofluorescence with monoclonal anti-Lyt 2 antibody as described in Fig. 1 legend and the % Lyt 2⁺ cells refers to cells staining above control samples stained with the second-step reagent alone.

examined in this study were beating spontaneously. They were made quiescent by addition of 2×10^{-7} M ACh to the bath solution before a pipette-membrane seal was established. The average resting potential V_r in these conditions was -63 ± 4 mV (mean $\pm$ s.e., 6 cells). The channel activity of the membrane patch under the pipette tip did not seem to be affected by application of ACh to the cell surface outside the patch.

Figure 1A demonstrates the current-voltage (I - V) relations of ACh-activated whole-cell currents in single S-A nodal cells in 5.4 mM K^+ and 20 mM K^+ Tyrode solution. The amplitude of the ACh-activated current was measured as the difference between currents recorded before and during the application of ACh. As shown in Fig. 1B, the ACh-dependent current relaxes at both the beginning and the end of a voltage step and reverses its polarity at the assumed V_K (K^+ current reversal potential, about -45 mV at 20 mM extracellular K^+). These characteristics of the current are quantitatively represented in the I - V relations measured at the beginning (dotted curves) and near the end of the voltage step (continuous curves) in Fig. 1A. Each pair of the curves crossed at about -80 mV (5.4 mM K^+) or at -45 mV (20 mM K^+). The inward-going rectification of the ACh-activated ion channel is obvious from both the

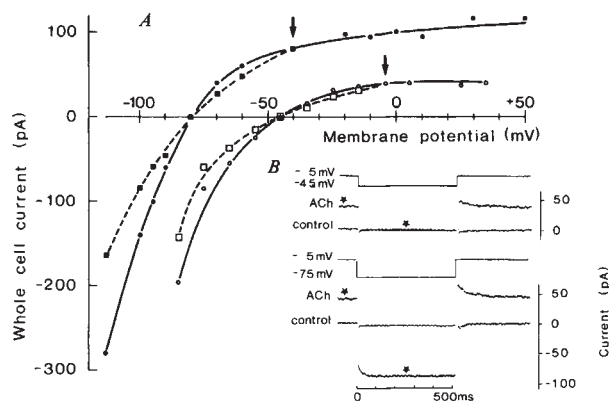


Fig. 1 **A**, Current-voltage relations of the ACh-activated K^+ current in dispersed S-A nodal cells. After enzymatic dispersion the cells were superfused with normal Tyrode solution for about 2 h. Many cells became round and the rate of formation and the stability of high-resistance pipette-membrane seals was much higher on rounded cells than on elongated cells. After establishing a 'gigaseal' the membrane patch was broken by a pulse of suction and the membrane potential was voltage-clamped. From the holding potential (arrows) the voltage was stepped for 500 ms to various more positive or negative potentials. The membrane current was recorded first before and then during application of ACh from a second ACh (0.1 mM)-containing pipette. The difference between the two current recordings (with and without ACh) is plotted at different membrane potentials. The current-voltage relations were obtained from two different cells bathed in 5.4 K^+ (closed symbols) and 20 K^+ (open symbols) Tyrode solution. The squares indicate the instantaneous currents, the round symbols represent the amplitude 500 ms later. **B**, Digitized traces of clamp currents in 20 mM K^+ bath solution in response to hyperpolarizing clamp steps from -5 mV to -45 mV (upper) and -75 mV (lower) membrane potential obtained in the absence (traces marked control) and in the presence of ACh (traces marked ACh and by asterisks). The time course of the voltage step is shown above the current traces. When in the presence of ACh the voltage is stepped to -45 mV, no net inward or outward current is elicited. The current traces obtained in the presence and the absence of ACh superimpose exactly, indicating that -45 mV is the zero-current potential for ACh-dependent currents in 20 mM K^+ Tyrode. The voltage step to -75 mV elicits, in the absence of ACh, a very small inward current of <10 pA. In the presence of ACh the large instantaneous inward current is followed by a relaxing current with a relaxation time of 17 ms. To avoid interference by the slow inward current in measuring the ACh-activated K^+ current, the voltage steps were made from the holding potential of -5 mV. The composition of pipette solution for whole-cell recording was (in mM): KAsp 100, KCl 20, EGTA 1, K-ATP 10. The pH was adjusted to 7.2 with 5 mM HEPES.

instantaneous and the steady-state I - V curves. These features of the ACh-dependent membrane current in dispersed single cells are qualitatively similar to those described for the multicellular preparation⁹.

The larger single channel current amplitude as well as the absence of delayed rectifying K^+ currents at potentials negative to V_K prompted us to measure ACh-dependent single channel currents predominantly at potentials negative to V_K (as inward currents). The K^+ concentration in the pipette solution was, therefore, elevated to 20 mM K^+ or higher. Figure 2A shows examples of brief current pulses recorded with a 20 mM K^+ pipette solution containing 2×10^{-7} M ACh. At the resting membrane potential (trace marked 0 mV), current steps are inward. When the patch membrane potential is changed with respect to V_r , the step size increases with hyperpolarization. Current steps reverse in sign on depolarization of the membrane patch by more than $+20$ mV. Figure 2B illustrates the distribution of outward (upper graph) and inward (lower graph) current step sizes at approximately the same absolute driving force. The strong rectification of the channel's conductance is indicated by the approximately five times larger step size when the current is inward. This behaviour of the single channel I - V relation is consistent with the rectification of the instantaneous I - V curve of the whole cell current shown in Fig. 1A. The reversal potential is about 20 mV more positive than the average resting potential of -63 mV. This can be accounted for by the elevated K^+ concentration in the pipette. The single channel chord conductances in these conditions were 25 pS for inward and 5 pS for outward current flow (best fit for the lines in Fig. 2C).

When the K^+ concentration in the pipette solution is increased from 20 to 70 mM or 140 mM, the average amplitude of ACh-activated inward currents recorded at V_r increases from 0.58 ± 0.04 pA to 1.8 ± 0.12 pA and 3.4 ± 0.4 pA, respectively. This K^+ concentration dependence shows that the ACh-activated channels are predominantly K^+ -selective. Using constant field assumptions, taking V_r as -63 mV and the intracellular K^+ concentration as 140 mM, a single channel K^+ permeability of the order of 10^{-13} cm³ s⁻¹ is obtained. This is in the same range as that of voltage-dependent axonal K^+ channels¹⁰.

The ACh concentration dependence of m-AChR channel activation was measured in 23 patches with pipettes containing 70 mM K^+ and at a patch membrane potential -20 mV more negative than the cell's resting potential. The mean patch current was measured as the average deviation of the current from the zero-current baseline (see, for example, Fig. 2A) during the first 60 s following the formation of a pipette-membrane seal. It increased approximately linearly with ACh concentration from -0.48 ± 0.2 pA at 10^{-7} M ACh to -3.1 ± 0.8 pA at 10^{-6} M ACh. The linear concentration dependence of the patch current is consistent with the apparent equilibrium constant of 1.7×10^{-6} M determined for the ACh-activated current in the multicellular preparation¹¹.

In the absence of ACh in the pipette, brief inward current steps of 1–2 ms average duration were measured in 6 of the 8 patches (Fig. 3A, inset). These 'resting' K^+ currents were similar in size and duration to the currents observed in the presence of low ACh concentrations (Fig. 4A, B, inset). The conductance of the resting K^+ channels at V_r and at 70 mM extracellular K^+ was 39 ± 4 pS compared with 35 ± 3 pS for ACh-activated channels. Their mean open time was 1.4 ± 0.2 ms (Fig. 3A) which is also comparable to the value of 1.8 ms for ACh-activated channels. They occurred, however, at much lower frequency. The mean patch current in the absence of ACh was -0.02 ± 0.007 pA, measured in the same conditions as the ACh-dependent patch current. Resting K^+ currents were also observed when ACh was omitted from both the bath and the pipette solution and also when the pipette contained 10^{-7} M atropine. All cells in which this 'nodal' class of resting K^+ currents was observed were ACh-sensitive.

Another class of resting K^+ currents was found in non-beating rounded cells or in elongated atrial cells. Examples are shown

Fig. 2 Conductance properties of single m-AChR channels. **A**, Examples of ACh-activated inward and outward current steps recorded from an A-V nodal cell at various patch membrane potentials. The current recording pipette contained the following solution (in mM): NaCl 120; KCl 20; CaCl₂ 4; Na-HEPES 5 and 2×10^{-7} M ACh. The numbers on the left of each trace indicate the shift in patch potential, ΔV , with respect to the cell's resting potential (V_r); for example '-20 mV' indicates a patch potential -20 mV more negative than V_r . Current steps reverse in polarity when the patch potential is made about 20 mV more positive than the resting potential. Inward current steps are downwards. The extrapolated reversal potential is about 20 mV more positive than V_r . All records are filtered at 0.8 kHz low pass (-3 dB). The continuous lines indicate the fitted zero-current baseline, the dotted line the average step size i at each potential. **B**, Distribution of outward current step-amplitudes at a membrane patch potential of +80 mV more positive than V_r (upper graph) and of inward current steps at patch potential -40 mV more negative than V_r (lower graph). The net driving force in both cases is about 60 mV. Both distributions are singly peaked with mean step sizes of 0.32 pA and 1.68 pA, respectively. **C**, Current-voltage relation of ACh-activated single channel currents obtained with 20 mM K⁺ in the pipette. The mean amplitude obtained from step size distributions as shown in **B** is plotted as a function of the patch potential. Zero mV shift in patch potential represents measurements of the cell's resting potential. The chord conductance of the m-AChR channel was 25 pS for inward current flow and 5 pS for outward current flow. The lines represent linear regression lines to the data points. The I - V relation for outward currents is fitted with the restriction of passing through the zero-current intercept extrapolated from the inward current data points (that is, 22 mV more positive than V_r).

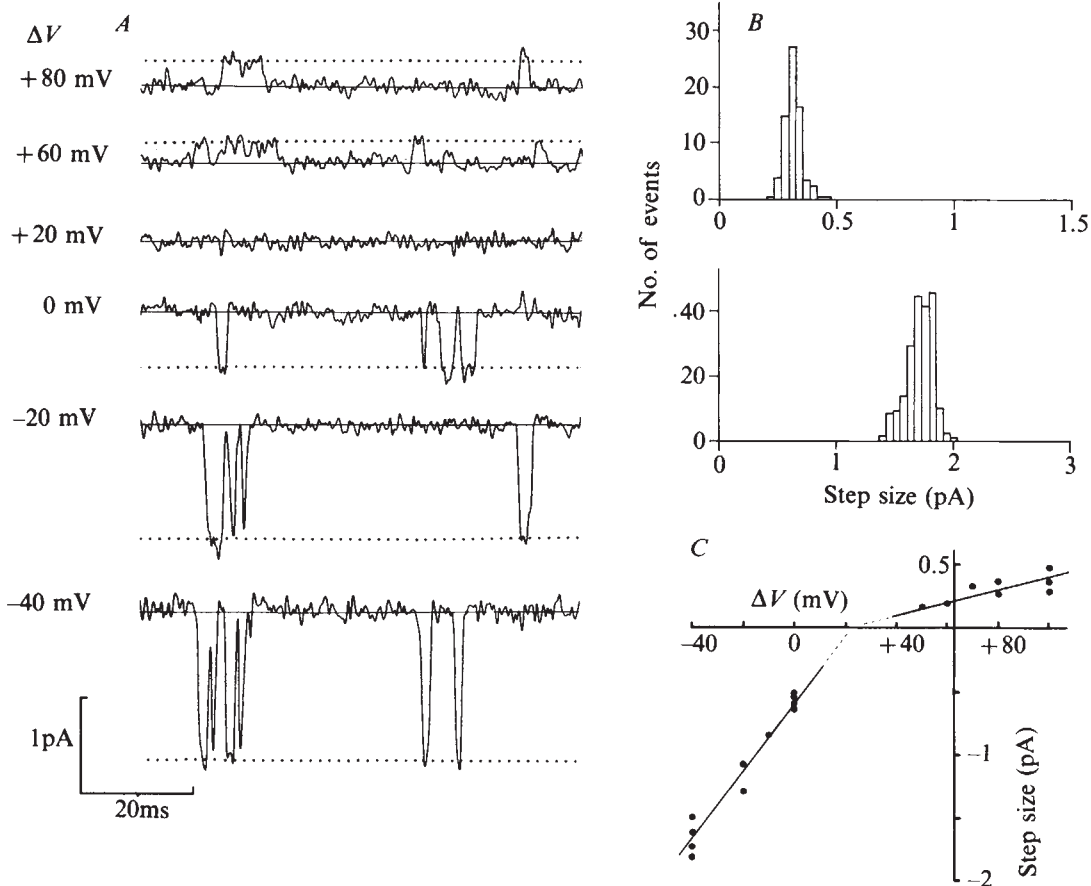
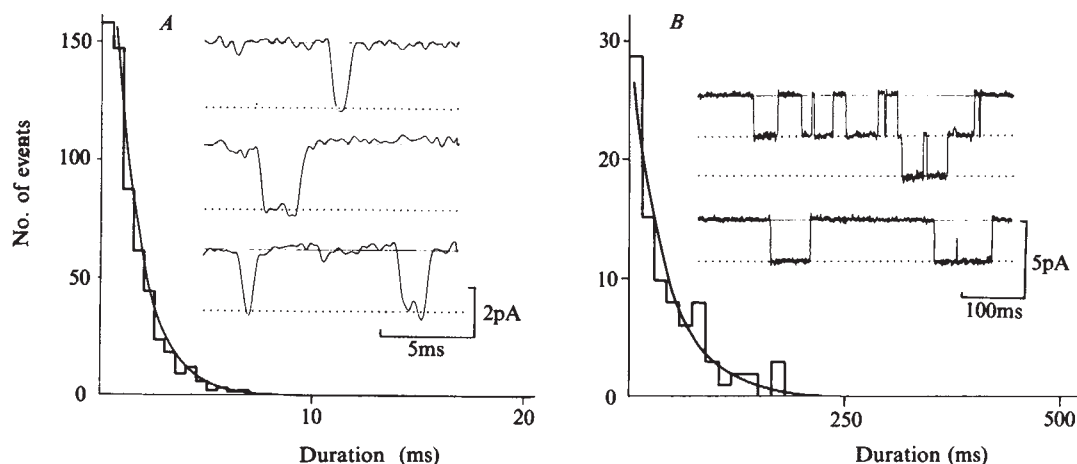


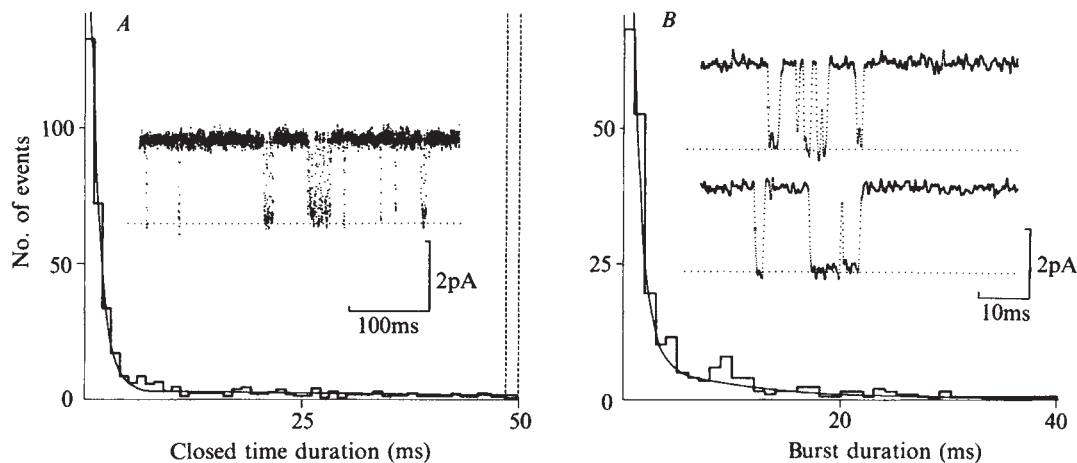
Fig. 3 Two classes of 'resting' K⁺ channels observed in single cells from dispersed A-V nodes. Recordings were made in the absence of ACh in the bath and the pipette solution. Bath solution was normal Tyrode solution. The pipette contained 70 mM K⁺. **A**, Recording from a rounded, non-beating A-V nodal cell. The resting K⁺ currents shown in the inset occur as short pulses of 2.4 pA amplitude at V_r - 20 mV. Records in the inset are filtered at 4 kHz low pass. The histogram represents the distribution of current pulse durations and is fitted (continuous line) by a single exponential with a time constant of 1.4 ms by minimizing chi-square. The origin of the fitted curve is off the scale (420 events). **B**, Recording from another rounded, non-beating cell. The resting K⁺ currents shown in the inset have an amplitude of 2.5 pA at V_r - 20 mV. The histogram of current pulse durations is fitted (continuous line) by a single exponential with time constant of 48 ms.



in the inset of Fig. 3B. The slope conductance of the channels mediating these currents was 34 ± 6 pS at V_r when measured with 70 mM K⁺ in the pipette solution. This value is only slightly smaller than that of the nodal resting K⁺ channel. Their mean open time, around 50 ms, was, however, much longer (Fig. 3B).

These currents also show strong inward rectification at potentials positive to V_K and they inactivate with membrane hyperpolarization at potentials negative to V_K . They resemble closely the resting K⁺ channels observed in ventricular cardiocytes¹². Cells in which this 'ventricular' class of resting K⁺ current was

Fig. 4 Bursts of single channel currents at low ACh concentrations. The patch pipette contained 70 mM K^+ and 2×10^{-7} M ACh. The bath solution was normal Tyrode solution. The membrane patch was hyperpolarized by -20 mV. **A**, The histogram shows the distribution of intervals between 1,021 single current pulses (that is, the closed time durations). Currents occur as single pulses or as bursts of pulses. The inset shows a digitized sample record at low time resolution. The distribution is fitted by the sum of a slow and a fast exponential component (continuous line) by minimizing chi-square. The respective time constants were 80 ms and 1.3 ms. The dotted bar in the last bin represents the number of intervals which have a duration longer than 50 ms and extends far off the scale (471 events). **B**, The histogram shows the distribution of current bursts as defined in the text. The inset shows digitized records of bursts of current pulses at higher time resolution. The distribution is fitted by the sum of two exponentials (continuous line) with time constants of 11 and 1.3 ms. The area under the fast component is 63% of the total area. The origin of the fitted curve is off scale (85 events). The critical duration for the interval between current pulses belonging to a burst was chosen as approximately four times the time constant of the fast component in the distribution of closed times (Fig. 4A).



recorded (probably corresponding to I_{K1} in conventional current measurements) were insensitive to ACh. Thus, pacemaker nodal cells are characterized by resting K^+ channels with a gating behaviour different from that of resting K^+ channels in non-beating atrial or ventricular cells.

The gating behaviour of ACh-activated channels was studied in the presence of 2×10^{-7} M ACh in the pipette. The inset of Fig. 4A shows a recording where elementary currents occur well separated at a low overall frequency (<10 s $^{-1}$). The opening of channels does not occur at random intervals since a large proportion of the currents is grouped into 'bursts' of current pulses (Fig. 4B, inset). The distribution of the intervals between channel openings is fitted by the sum of two clearly separated components of 1.3 ms and 80 ms average duration in this experiment (Fig. 4A). When a burst of currents is defined as any sequence of current pulses separated by intervals not larger than a critical duration (6 ms), the distribution of burst durations shown in Fig. 4B is obtained. It is fitted by the sum of two exponential components of 11 ms and 1.1 ms duration. From the area under the slower component in the distribution of burst durations and the number of short closed times we calculate that a burst consists on average of 5.3 current pulses separated by gaps of 1.3 ms average duration in this experiment. Comparable results were obtained in three other patches.

Current bursts could represent the reopening of a m-AChR channel during a single occupancy as inferred previously for the nicotinic AChR channel¹³. Assuming a two-step reaction underlying m-AChR channel activation¹⁴



where R and AR represent the closed and AR* the open channel, the estimates of the rate constants $k_{-1} = 150$ s $^{-1}$, $\beta = 460$ s $^{-1}$ and $\alpha = 510$ s $^{-1}$ are obtained (means from four patches) from the average number and duration of the closing gaps during a burst and from the burst duration^{15,16}. According to this analysis the rates of channel opening and closing are nearly one order of magnitude larger than previously assumed⁴. The reaction scheme above predicts an excess of short openings in the distribution of burst durations^{15,16} as is observed. However, the measured fraction of short openings, about 60% of all events, is far larger than expected ($<5\%$). The mechanism underlying this excess of short openings is as yet unclear. They could, for example, represent openings of K^+ channels coupled to a different class or state of m-AChRs with a lower affinity¹⁷.

The resting K^+ currents observed in some nodal cells could represent spontaneous openings of m-AChR channels. The

two-step reaction scheme describing the burst-like activation of K^+ channels by ACh in pacemaker cells is similar to that inferred for the opening of endplate channels in skeletal muscle¹³. The main difference between nicotinic and muscarinic AChR channels lies in the considerably smaller reaction rates governing m-AChR channel activation.

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Common site of integration of HTLV in cells of three patients with mature T-cell leukaemia-lymphoma

Beatrice Hahn, Vittorio Manzari, Sandra Colombini, Genoveffa Franchini, Robert C. Gallo & Flossie Wong-Staal

Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

Human T-cell leukaemia-lymphoma virus (HTLV) was first isolated in the United States from a patient with an aggressive form of cutaneous T-cell lymphoma¹ (CTCL) and was later found associated with clusters of adult T-cell leukaemia-lymphomas (ATL) in various parts of the world, including Japan and the Caribbean^{2–4}. Leukaemic cells of the HTLV-positive patients seem to be clonal expansions of single infected cells since the provirus(es) are found at the same sites in a given

patient^{9,10}. In avian leukosis virus-induced B-cell lymphomas, the provirus very frequently integrates at several discrete sites in a common domain near the cellular gene, *c-myc*, that it activates^{11,12} and it has been speculated that the same would hold true for other chronic leukaemia viruses. We report here that cultured cells from two US patients with CTCL and fresh leukaemia cells of a Japanese patient with ATL contained an HTLV provirus integrated at the same site. In addition, a cord blood T-lymphocyte cell line established by co-cultivation with one of the two HTLV-positive CTCL cell lines¹³ also contained HTLV provirus contiguous with the same flanking cellular sequences. Ten other HTLV-positive cell samples did not show integration of HTLV at this site, suggesting that there is more than one discrete site of HTLV integration in tumour cells.

We have recently cloned a defective provirus and flanking cellular sequences from an HTLV-positive neoplastic T-cell

line CR-M2 derived from the first US patient¹⁴. In the experiments described here, a 1.8-kilobase (kb) fragment (CR_{S2}) containing unique flanking cellular sequences was used to probe other HTLV-infected and uninfected cell lines. The rationale is that if there is a common locus of cell sequences into which HTLV preferentially integrates, one should see at least one allelic copy of this sequence modified by the provirus insertion. This would result in additional restriction enzyme bands which should co-migrate with some viral sequences. CR-M2 contains three copies of HTLV provirus, one of which is represented by the clone CR-1 (ref. 14). A simplified genetic map of CR-1 is inserted in Figs 1–3, showing a 9.8-kb DNA fragment containing HTLV 3' LTR-*env-pol* sequences flanked by cellular sequences on both sides. The two probes used were CR_{S2}, a 1.8-kb *Sst*I fragment containing only cell sequences 3' of the HTLV provirus, and CR_{CH}, generated by a *Cla*I-*Hind*III diges-

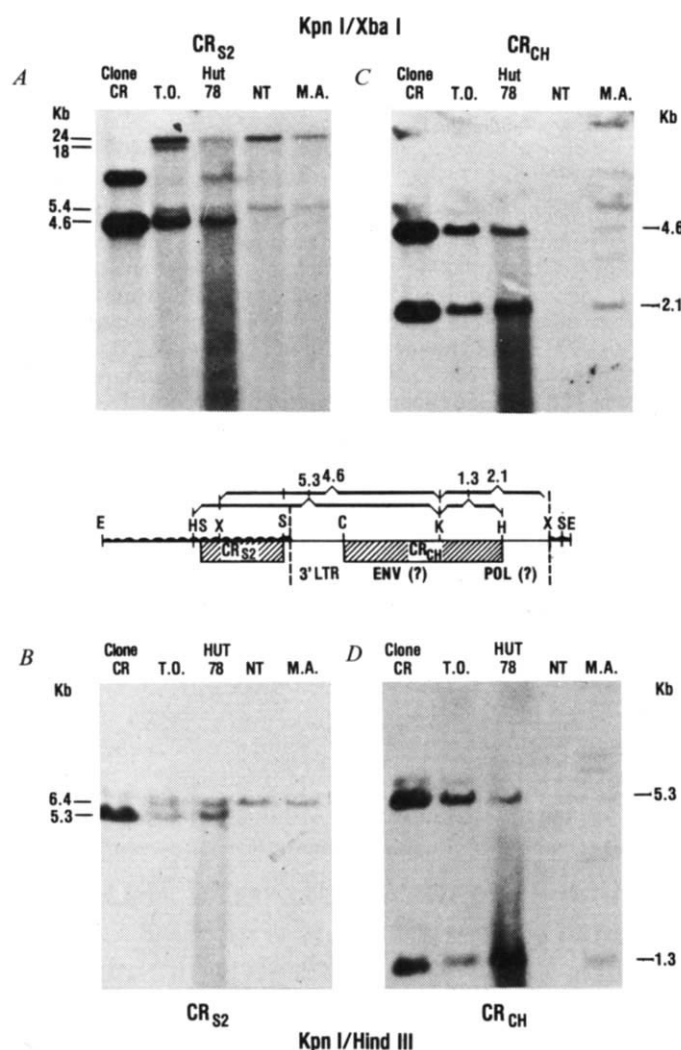


Fig. 1 Restriction analysis of cellular (CR_{S2}) and viral sequences in DNA from normal and HTLV-positive neoplastic cells. DNA was extracted from normal human thymus and leukaemic cells of an ATL patient (T.O.), a neoplastic T-cell line (HUT-78), and skin biopsy of a CTCL patient (M.A). DNA from the clone CR-1 was used as control. DNA was digested with the enzymes *Kpn*I and *Xba*I, or with *Kpn*I and *Hind*III, size-fractionated on agarose gels and transferred to nitrocellulose filters²⁰. Duplicate samples were hybridized in parallel to CR_{S2} (A, B) or CR_{CH} (C, D) (HTLV *pol-env*) probes. One or two CR_{S2}-specific fragments were detected in all DNAs from both normal and neoplastic cells. In addition, bands detected with both CR_{S2} and CR_{CH} were detected in T.O. and HUT-78 DNA. A simplified restriction map of clone CR-1 is shown for reference. The vertical dotted lines delimit the viral sequences and the black wavy lines represent flanking cellular sequences.

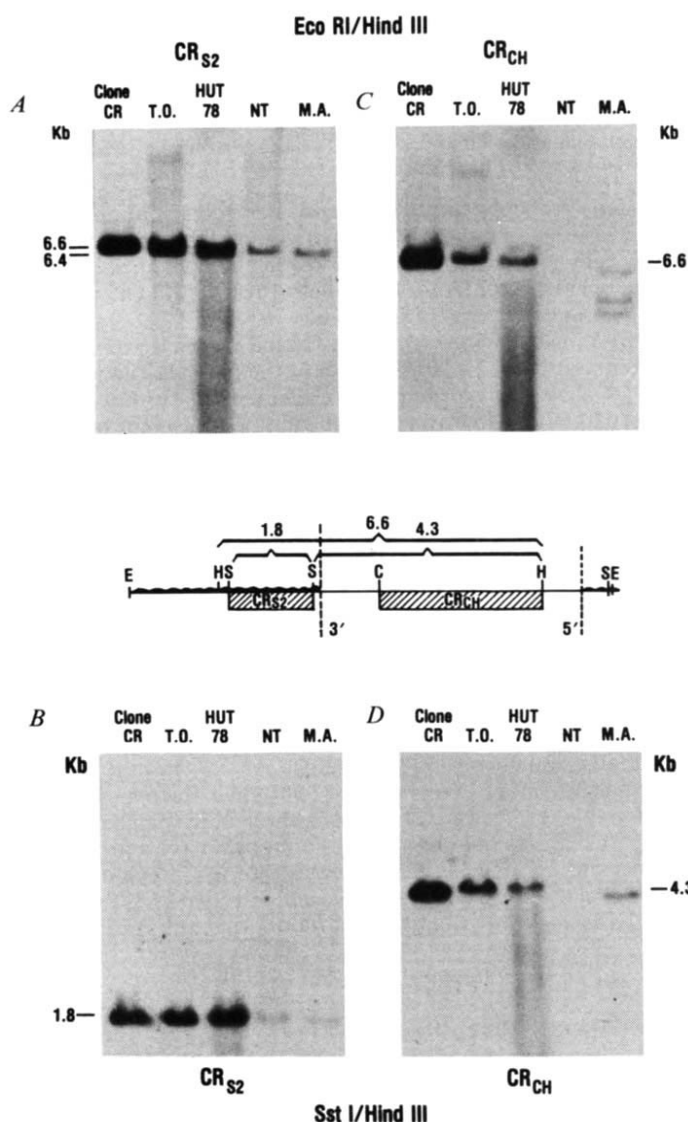


Fig. 2 Further restriction analysis of cellular and viral sequences in DNA from normal and HTLV-positive neoplastic cells. Source of DNAs and experimental procedures as for Fig. 1. Co-digestions with *Eco*RI and *Hind*III, or *Sst*I and *Hind*III were carried out. The *Eco*RI/*Hind*III digestion yielded a doublet of 6.6 and 6.4 kb for clone CR, T.O. and HUT-78 DNA when hybridized to CR_{S2} (A). One of these (6.6 kb) also hybridized to CR_{CH}. Unlike the other enzymes tested, *Sst*I digestion unlinked viral and cellular sequences in T.O. and HUT-78 DNA as in the original clone CR-1.

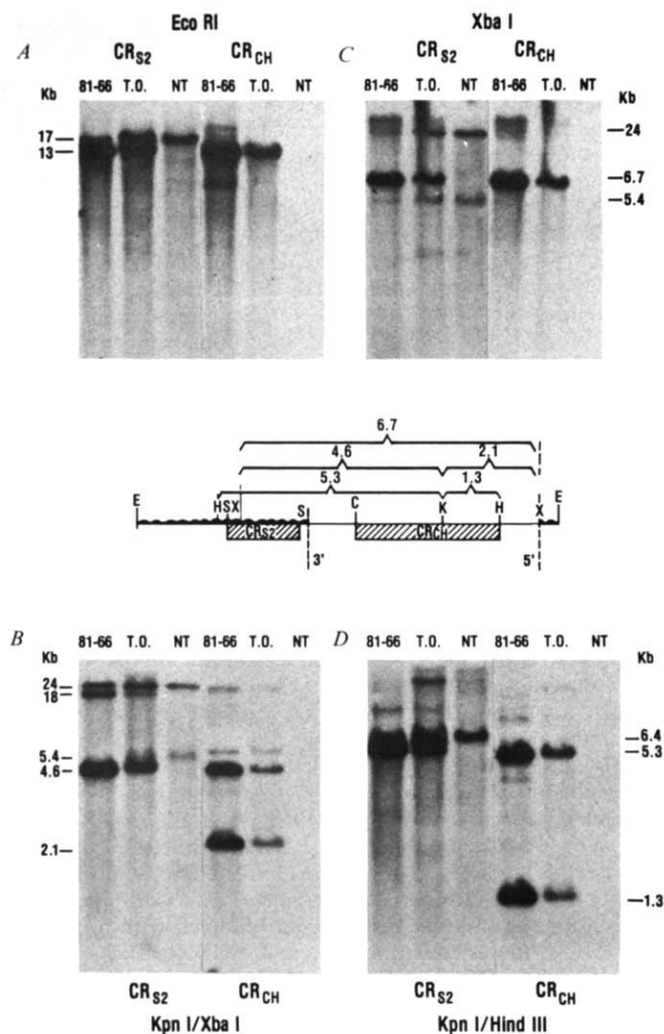


Fig. 3 Evidence for amplification of contiguous cellular (CR_{S2}) and viral sequences in a cord blood T-lymphocyte cell line infected with HTLV. Experimental details as for Fig. 1. 81-66 is a cell line derived from normal cord blood T-lymphocytes transformed *in vitro* by co-cultivation with the HTLV-positive neoplastic cells CR-M2 (ref. 14). 30 μ g of DNA were used in each lane.

tion and containing HTLV *pol-env* sequences. Hybridization of CR_{S2} to a variety of human DNAs generally showed a constant restriction enzyme pattern. These samples included DNA from many normal tissues, leukaemic cells and 10 HTLV-positive cell lines or fresh cells. Two examples, a normal human thymus (NT) and a skin tumour biopsy of a patient (M.A.) with HTLV-positive diffused mixed lymphoma are shown in Figs 1 and 2 (panels A and B). Two DNA samples showed additional bands with CR_{S2} with many enzymes tested. These samples were obtained from fresh (uncultured) leukaemic blood cells of a Japanese ATL patient (T.O.) positive for HTLV and from a cell line, HUT-78, derived from a US patient with Sézary syndrome¹⁵. HUT-78 was a mature T-cell line originally thought to be HTLV-negative because it expressed no detectable HTLV antigen or mRNA. However, analyses of its DNA with cloned HTLV probes revealed a defective integrated provirus (R.C.G. *et al.*, in preparation). As shown in Figs 1A, B and 2A, DNA from T.O. and HUT-78 digested with different restriction enzyme combinations revealed DNA bands with CR_{S2} that are additional to bands in common with normal thymus and M.A. DNA. To determine whether the extra bands were cell-viral junction bands, duplicate filters were hybridized to CR_{CH} . As shown in Figs 1C, D and 2C, normal thymus DNA does not contain sequences hybridizable to CR_{CH} . In all cases, the extra CR_{S2} bands in T.O. and HUT-78 DNA co-migrated

with a viral band. Furthermore, integration of HTLV in T.O. and HUT-78 occurred at the same site as in CR-1 since all the 3' junction bands were the same size in all three DNAs. Digestion with *SstI/HindIII* released a 1.8-kb fragment from T.O. and HUT-78 DNA the same size as CR_{S2} which is unlinked to viral sequences (Fig. 2B, D).

We also examined a cord blood T-lymphocyte cell line, 81-66, infected with HTLV by co-cultivation with CR-M2 cells¹⁴. DNA from 81-66 cells also showed extra CR_{S2} bands which co-migrated with viral bands (Fig. 3). The results were also consistent with precisely the same location of the provirus in T.O. and HUT-78 as in CR-1. Moreover, the extra CR_{S2} bands were much stronger than the common CR_{S2} bands, suggesting that the modified cellular locus might have been amplified. The intensity of the viral bands in 81-66 compared with T.O. and HUT-78 was also consistent with amplification of the locus where HTLV sequences have been inserted. However, the size of the amplification unit has not been determined. It is not clear whether transmission of HTLV to 81-66 cells was through infectious virus particles, in which case it would represent another independent integration at the same site as CR-1. Alternatively, transmission might have occurred by fusion of a segment of CR-M2 DNA containing HTLV and the neighbouring cellular sequences into 81-66 chromosomal DNA.

Cord blood T-lymphocytes derived from normal donors but subsequently infected with HTLV *in vitro* resemble primary HTLV-positive neoplastic T cells in their immortalized growth, partial or complete independence of T-cell growth factor, and induction of specific antigens and cell morphology^{16,17}. Therefore, the cells may be considered 'transformed'. However, in spite of its capacity to transform cells *in vitro*, paradoxically, HTLV probably does not carry a transformation-specific (*onc*) gene. As noted earlier, HTLV-positive tumour cells are clonally derived. Clonality of the tumour cells is a common feature among tumours induced by the chronic leukaemia viruses that lack *onc* genes. Our present data indicate that at least three independent cell samples obtained from patients in the United States or Japan contained HTLV integrated in the same immediate locus, suggesting that integration next to specific cellular genes is important in neoplastic transformation. Failure to detect insertion of HTLV at precisely the same site in 10 other HTLV-positive cell samples means that there is more than one discrete site into which HTLV integrates in different tumour cells. These sites may or may not be present in a single larger domain. Our results with HTLV integration sites mimic those of avian leukaemia virus in chicken B-cell lymphomas^{11,12}, and the most recent observation with mouse mammary tumour virus-induced mouse mammary tumours¹⁸ and Moloney murine leukaemia virus-induced rat lymphomas¹⁹. Hybridization of S2 to known viral *onc* genes failed to show any homology. Furthermore, the S2 locus has been mapped to a human chromosome not known to contain any cellular *onc* gene homologues (G.F. *et al.*, unpublished). It will be important to determine if any cellular genes are activated transcriptionally by HTLV insertion, and if so, the nature of such genes.

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Note added in proof: We have analysed 17 more HTLV-positive samples and have found an additional fresh ATL cell sample that contained HTLV integrated at the S2 locus.

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Isolation of genetic elements that increase frequencies of plasmid recombinants

Anthony A. James*, Paul T. Morrison & Richard Kolodner*

Laboratory of Molecular Genetics, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115, USA

* Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115, USA

Some plasmid DNAs, when maintained in wild-type *Escherichia coli* strains, form high levels of oligomeric species while others remain primarily monomers^{1–9}. One explanation of this observation is that the plasmids that do not form circular oligomers lack a DNA sequence necessary for the formation or maintenance of circular oligomeric species. Here we describe the isolation of segments of DNA from the *E. coli* genome and other sources that through a *recA*⁺-dependent process: (1) stimulate the conversion of monomeric plasmids to different oligomeric forms, (2) stimulate the conversion of an oligomeric plasmid to a mixture of monomeric and different oligomeric forms, and (3) increase the frequency of recovery of figure-8 molecules. Both *cis*-acting and *trans*-acting elements were found. These elements seem to act by stimulating either the frequency of the recombination events that lead to the interconversion of different oligomeric plasmid DNA molecules or some process involved in the maintenance of newly-formed recombinant molecules.

The criterion for identifying a recombinogenic element was the *recA*⁺-dependent ability of an element to cause a plasmid to form high levels of circular oligomers. To identify elements in *E. coli* chromosomal DNA, random *EcoRI* fragments were inserted into the single *EcoRI* site of the plasmid pVH51 (ref.

9) and the resulting plasmids used to transform an *E. coli recA* strain to colicin-E1 resistance. Transformants with monomeric plasmids each containing a single insert 2–4 kilobases (kb) long were identified. These plasmids were tested for their ability to form circular oligomers by transformation of a wild-type *E. coli* strain and subsequent examination of the plasmid DNA for the presence of circular oligomers using agarose gel electrophoresis. Figure 1 illustrates the ability to form circular oligomers of the cloning vector pVH51 (Fig. 1, lanes a, b), a hybrid plasmid pRDK115 lacking a recombinogenic element (Fig. 1, lanes c, d), and a hybrid plasmid pRDK101 containing a recombinogenic element (Fig. 1, lanes e, f) when present in isogenic *recA* and wild-type *E. coli* strains. In two independent experiments ~20% of the hybrid plasmids formed circular oligomers. We also determined that a *HindIII* fragment of the Lac promoter-operator region¹⁰, Lac 789 (L₈UV₅), had recombinogenic activity when inserted into pVH51. In addition, the plasmids pML21⁹ and pACYC184¹¹ showed recombinogenic activity compared with their respective parental plasmids, pVH51 and p15A.

We were interested in determining if recombinogenic elements could also facilitate intramolecular recombination. Circular tetramers of pVH51 and pRDK101 were isolated using agarose gel electrophoresis^{5,6} and analysed as described above for their ability to undergo intramolecular recombination. Circular tetramers of pVH51 were stable in *recA* strains and were inefficiently converted to smaller circular oligomers and monomers in wild-type strains (Fig. 2, lanes b, c) and *recB recC* strains (not shown), indicating that the failure of pVH51 to form oligomers was not due to the rapid breakdown of oligomers or the inability of wild-type strains to maintain oligomers. Circular tetramers of pRDK101 were stable in *recA* strains (Fig. 2, lane d) but were converted to a mixture of different circular oligomers and monomers in a wild-type strain (Fig. 2, lane e). Similar results were obtained with tetramers of pRDK43 (data not shown) and pACYC184⁶. Thus, recombinogenic elements stimulate intramolecular as well as intermolecular recombination events.

Agarose gel electrophoresis distinguishes only poorly between circular oligomers, catenated oligomers and figure-8 molecules. Electron microscopy was therefore used to quantify these different DNA species in preparations of pVH51, pRDK101, p15A and pACYC184 purified from wild-type *E. coli* strains (Table 1). The results showed that pRDK101 DNA preparations contained 8 times as many circular oligomers as were present in the parental pVH51 DNA preparations while pACYC184 DNA preparations contained 90 times as many circular oligomers as the parental p15A DNA preparations. Catenated oligomers and figure-8 molecules were only present at low levels.

Figure-8 molecules have been proposed to be recombination intermediates of circular genomes because they have many of the properties expected of a Holliday recombination intermedi-

Table 1 Frequencies of different plasmid DNA species in wild-type *E. coli* (%wt)

Plasmid	Monomer	Dimer	Trimer	Tetramer	<i>n</i> > 4	Other*	N†	Figure-8s
pVH51‡	92.7	5.8	0.9	<0.1	<0.1	0.6	928	0.02
pRDK101‡	47	39	6	3	2	2.7	581	0.12
pRDK43‡	79.9	17.4	1.1	0.38	0.05	1.2	1,896	0.08
p15A§	98.9	0.7	<0.2	<0.2	<0.2	0.4	593	0.01
pACYC184§	5	72	13	5	<0.2	5	442	0.09

Plasmid DNA preparations were digested with *KpnI* (pVH51, pRDK43, pRDK101), *BglI* (p15A) or *EcoRI* (pACYC184) at 10°C for 10 min, using the buffer conditions provided by New England Biolabs, to convert the circular molecules to linear monomers and X-forms in conditions which limit branch migration, before mounting for electron microscopy^{6,7,19}. The frequencies of figure-8 molecules were calculated as described elsewhere⁶. Between 7,000 and 12,000 molecules were counted for each determination.

* Catenanes, figure-8s.

† Total number of molecules analysed.

‡ Purified from *E. coli* RK1128.

§ Purified from *E. coli* C600 *leu*⁻, *lac*⁻, *thr*⁻, *su*⁺, *Tl*^r.

ate^{2,4,12-14}. To quantify the figure-8 molecules present in wild-type strains, DNA preparations were converted to a mixture of X-forms and linear monomers by digestion with a restriction endonuclease having one cleavage site per monomer unit and analysed by electron microscopy. X-form molecule frequencies were converted to figure-8 molecule frequencies using a procedure described in ref. 6. The results indicated that DNA preparations of the recombinogenic plasmids pRDK43, pRDK101 and pACYC184 contained 5–10-fold more figure-8 molecules than the parental plasmid DNAs (Table 1).

The ability of a recombinogenic element to stimulate recombination in *trans*, was tested to provide insight into a possible mechanism of action. To determine if the recombinogenic

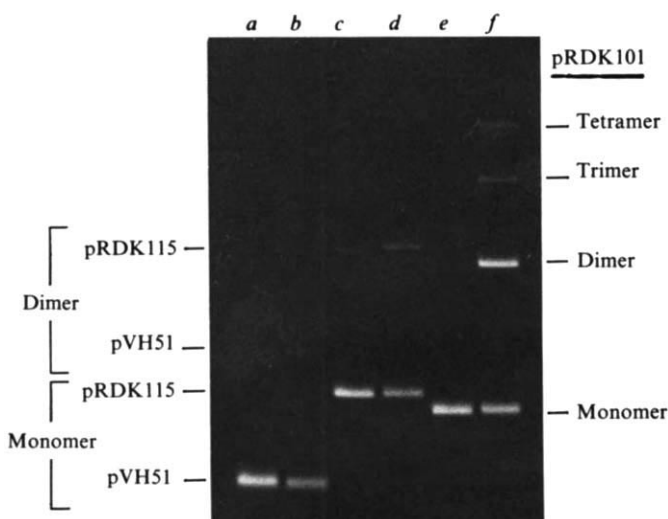


Fig. 1 Electrophoretic analysis of pVH51 DNA and derivatives containing inserts of *E. coli* DNA purified from isogenic *recA* and wild-type *E. coli* strains. pVH51 DNA⁹ was purified as previously described⁶ and *E. coli* JC5469⁸ chromosomal DNA was the gift of J. Joseph of this laboratory. In separate reactions, pVH51 DNA and *E. coli* DNA were digested with *EcoRI* in conditions supplied by the manufacturer (New England Biolabs). The DNAs were then purified by phenol extraction and ethanol precipitation²⁰ and the resulting DNA was suspended in TE buffer (10 mM Tris, 1 mM EDTA pH 8.0). The *E. coli* fragments were then joined to the linear pVH51 DNA in a two-stage reaction with T4 DNA ligase. The first reaction contained 0.5 µg pVH51 DNA, 2.5 µg *E. coli* DNA, 40 mM Tris pH 7.8, 5 mM β-mercaptoethanol, 8 mM MgCl₂ and 0.4 PP_i-exchange units²¹ of T4 polynucleotide ligase per ml in a final volume of 35.7 µl. After incubation at 12.5 °C for 30 min the volume of the reaction was increased to 1.19 ml with the buffer conditions adjusted to be identical to those of the first stage reaction and the ligase concentration increased to 2 units per ml. After incubation at 12.5 °C for 2 h the reaction was stopped by addition of 0.1 M EDTA pH 8.0 and 10 mg ml⁻¹ *E. coli* B tRNA to final concentrations of 10 mM and 20 µg ml⁻¹, respectively, and the nucleic acids were purified by phenol extraction and ethanol precipitation²⁰. The resulting hybrid pVH51-*E. coli* plasmids were then used to transform *E. coli* HB101 *recA13*, *hsdR*⁻, *hsdM*⁻, *lac24*, *leuB6*, *proA2*, *thi1*, *str*^r, *supE44* to colicin resistance²² and the resulting transformants were screened for the presence of monomeric plasmid DNAs containing a single inserted *EcoRI* fragment of *E. coli* DNA using standard screening procedures²³. Samples of monomeric pVH51 and hybrid pVH51-*E. coli* DNAs were then purified by preparative electrophoresis in agarose gels and used to transform *E. coli* W3110 *recA1*, *hsdR*⁻, *thyA*⁻, *rif*^r (RK1058) and W3110 *hsdR*⁻, *thyA*⁻, *rif*^r (RK1128) to colicin resistance²². The transformants were then analysed for the presence of different oligomeric forms of the input plasmid DNAs by electrophoresis on 0.5% w/v agarose gels as previously described⁶. Lane a, pVH51 DNA purified from a *recA* strain. Lane b, pVH51 DNA purified from a wild-type strain. Lane c, pRDK115 DNA purified from a *recA* strain. Lane d, pRDK115 DNA purified from a wild-type strain. Lane e, pRDK101 purified from a *recA* strain. Lane f, pRDK101 purified from a wild-type strain.

element present in pACYC184 could act in *trans*, pACYC184 and the compatible non-recombinogenic plasmid, pVH51, were used individually or together to transform an isogenic pair of *recA* and wild-type *E. coli* strains. Plasmid DNA was isolated from the transformants and analysed by agarose gel electrophoresis to detect circular oligomers of either pVH51 DNA or pACYC184 DNA. The results showed that pACYC184 was converted to a mixture of circular oligomers in a *recA*⁺-dependent reaction both in the absence (Fig. 3, lane b) and presence of pVH51 (Fig. 3, lane e) while pVH51 remained primarily monomers in both the absence (Fig. 3, lane d) and presence (Fig. 3, lane e) of pACYC184. Thus, the recombinogenic element present in pACYC184 can only act in *cis*. By inserting the *EcoRI* fragments containing recombinogenic elements into the *EcoRI* site of pACYC184 and testing these hybrid plasmids in experiments identical to those of pACYC184 alone, it was possible to determine if other recombinogenic elements could stimulate recombination in *trans*. These data demonstrated that the recombinogenic elements present in pRDK43 and the Lac 789 (L₈UV₅) fragment act in *cis* when inserted into pACYC184 (data not shown). The plasmid pRDK116, made by inserting the *E. coli* genomic fragment present in pRDK101 into pACYC184, was found to increase markedly the level of pVH51 circular oligomers when pRDK116 and pVH51 were maintained together in a wild-type strain (Fig. 3, lane k). Thus the recombinogenic element present in pRDK101 can act in

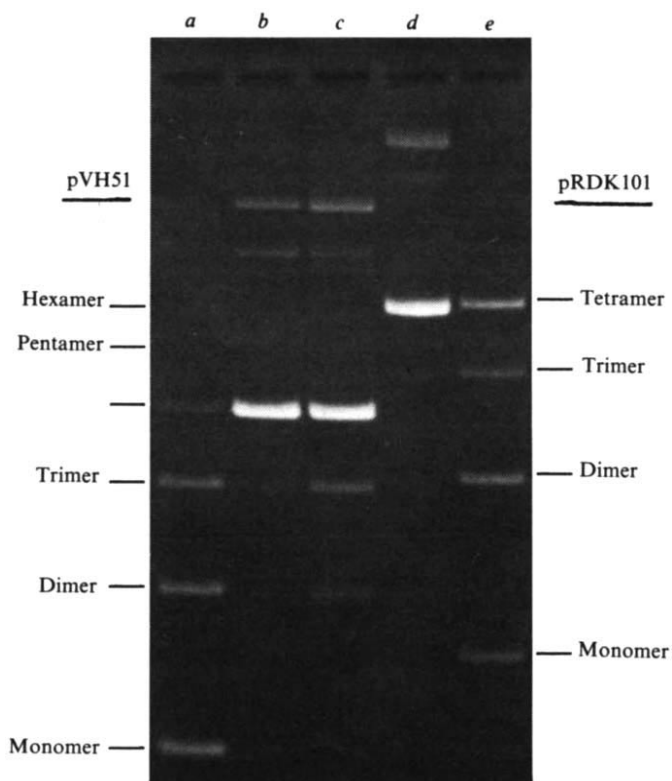


Fig. 2 Electrophoretic analysis of intramolecular recombination of tetrameric pVH51 and pRDK101 DNAs. Circular tetramers of pVH51 and pRDK101 were constructed essentially as described previously^{5,6} and stably maintained in *E. coli recA* strains. Tetrameric plasmid DNAs were purified from these *E. coli* strains and analysed for their ability to be converted into other oligomeric forms after transformation into *E. coli* RK1058 and *E. coli* RK1128 as described in Fig. 1 legend. Lane a, pVH51 oligomer markers purified from *E. coli* JC9604 containing an *sbcA* mutation⁷. Lane b, pVH51 tetramer DNA purified from a *recA* strain. Lane c, pVH51 tetramer DNA purified from a wild-type strain. Lane d, pRDK101 tetramer DNA purified from a *recA* strain. Lane e, pRDK101 tetramer DNA purified from a wild-type strain.

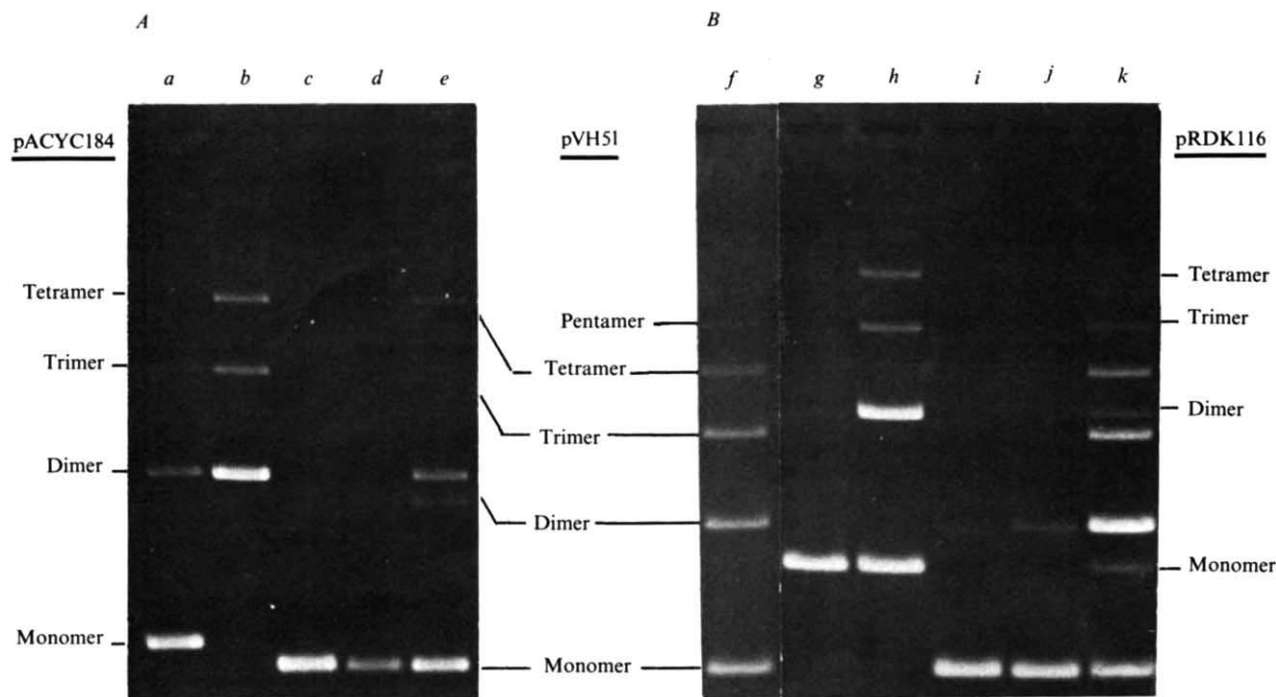


Fig. 3 Electrophoretic analysis of the stimulation of plasmid recombination in *cis* and *trans*. **A**, Monomeric pACYC184 plasmid DNA (tetracycline-resistant) and monomeric pVH51 plasmid DNA (colicin EI-resistant) were used to transform *E. coli* RK1058 and *E. coli* RK1128 either individually or together and the resulting transformants analysed as described in Fig. 1 legend. Lane *a*, pACYC184 DNA purified from a *recA* strain. Lane *b*, pACYC184 DNA purified from a wild-type strain. Lane *c*, pVH51 DNA purified from a *recA* strain. Lane *d*, pVH51 DNA purified from a wild-type strain. Lane *e*, plasmid DNA purified from a wild-type strain transformed with both pVH51 and pACYC184 monomer DNA. **B**, The plasmid pRDK116 was constructed by inserting the 2.1-kb insert contained in pRDK101 into the single *Eco*RI site of pACYC184 using a method similar to that described in Fig. 1 legend. Monomeric pRDK116 DNA (tetracycline-resistant) and pVH51 (colicin EI-resistant) were then used to transform *E. coli* RK1058 and *E. coli* RK1128 either individually or in pairs and the resulting transformants analysed as described in Fig. 1 legend. Lane *f*, pVH51 oligomer markers isolated from *E. coli* JC9604. Lane *g*, pRDK116 DNA isolated from a *recA* strain. Lane *h*, pRDK116 DNA isolated from a wild-type strain. Lane *i*, pVH51 isolated from a *recA* strain. Lane *j*, pVH51 DNA isolated from a wild-type strain. Lane *k*, plasmid DNA purified from a wild-type strain transformed with both monomeric pVH51 and pRDK116 DNAs.

trans when cloned together with the *cis*-acting element in pACYC184.

Our results indicate that certain DNA segments can dramatically facilitate recombination of certain plasmid DNAs when the plasmids contain these DNA segments. The effect of these DNA segments can be substantial, with the increase in the levels of circular oligomers observed ranging over 8–90-fold. The exact effect of these elements on the rate of plasmid recombination has not been determined. The presence of these DNA segments also leads to a 4–9-fold increase in the levels of figure-8 molecules. As most circular oligomers are formed by homologous recombination events and figure-8 molecules have been implicated as an intermediate in the process, it seems likely that these recombinogenic elements either increase the levels of plasmid recombination or increase the recovery of recombinant DNA molecules. Interestingly, both the *cis*- and *trans*-acting elements produce the same result, an increase in the interconversion of oligomeric forms.

A recent genetic analysis has demonstrated that plasmid recombination in *E. coli* strains containing *sbca* mutations no longer requires the *recA* and *recF* gene products and seems to occur via a recombination pathway which resembles the bacteriophage λ Red pathway^{6,15}. Because pVH51, pRDK101 and pACYC184 all form circular oligomers in *E. coli* strains containing *sbca* mutations (refs 6, 7 and Figs 2, 3) it seems likely that these recombinogenic elements are specific for the *recA*- and *recF*-dependent recombination pathway which occurs in wild-type *E. coli* strains.

The extent of homology between the different *cis*-acting recombinogenic elements and their mechanism of action are

unknown. It is tempting to think that the *cis*-acting elements are recombination hotspots. One such type of DNA sequence, Chi, is known to be present in *E. coli* DNA and has been extensively characterized^{16,17}. Two lines of evidence indicate that the *cis*-acting elements are not necessarily Chi and that they function differently from Chi. Computer analysis of the DNA sequence of the Lac 789 (L_{8UV5}) DNA fragment (A. Maxam, personal communication) containing a recombinogenic element, indicated that it did not contain the 8-base pair sequence of Chi¹⁷. Furthermore, Chi activity requires functional *recB* and *recC* genes¹⁶ which are not required for the activity of the recombinogenic elements described here⁶, indicating mechanistic differences between the two types of elements.

The *trans*-acting recombinogenic element could affect recombination by several different mechanisms which we are currently examining. The 2.1-kb insert containing the recombinogenic element is large enough to encode a protein that at high levels could affect plasmid recombination. Genetic analysis⁶ has demonstrated that this DNA segment does not complement *recA*, *recB* *recC* or *recF* mutations, making it unlikely that it is one of these genes. This DNA sequence on a high copy-number plasmid might bind completely a regulatory protein whose absence could induce or derepress the synthesis or alter the activity of proteins involved in plasmid recombination or the recovery of plasmid recombinants. This latter possibility is intriguing because several components of the RecF recombination pathway are subject to repression by the *lexA* gene product (ref. 18 and R.K., unpublished results).

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High-frequency precise excision of the *Drosophila* foldback transposable element

Mary Collins & Gerald M. Rubin

Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, Maryland 21210, USA

Precise excision of transposable elements in prokaryotes is a rare event which occurs at a significantly lower rate than transposition and other element-mediated events¹. Thus, we were intrigued by a eukaryotic transposable element which seemed capable of precise excision at high frequencies. The *white-crimson* (w^c) mutation in *Drosophila*, a highly unstable allele of the X-linked eye colour locus, *white*², resulted from the insertion of a member of the foldback (FB) transposable element family^{3,4}. This mutation reverts to its parental phenotype at a frequency of greater than 1 in 10³ X chromosomes². Characterization of these revertants by Southern blots of genomic DNA indicated that they resulted from loss of the w^c insertion³. Here we report the nucleotide sequence of the excision point in these revertants, and conclude that the FB element responsible for the w^c mutation is capable of precise excision at high frequencies.

The w^c allele was originally isolated as a partial phenotypic revertant of *white-ivory* (w^i), a mutant allele of *white* which resulted from the duplication of 2.9 kilobases (kb) of DNA within the *white* locus⁵. The w^c mutation was caused by the insertion of a 10-kb FB transposable element into this duplication^{3,4}. FB elements are a family of dispersed, repetitive DNA sequences with long, inverted repeats at their termini^{6,7}. These inverted repeats vary in length, and have an irregular subunit structure, which consists of a series of small, imperfect direct repeats^{7,8}. The terminal repeats in the FB element responsible for the w^c mutation are 2.2 and 3.4 kb long, and are separated by a sequence of ~4 kb which is not homologous to the inverted repeats, and which is present in only a few copies in the *Drosophila* genome⁴.

We have shown previously that two phenotypic classes of derivatives of the unstable w^c allele are associated with excision of the insertion³. Reversion of w^c to a wild-type phenotype occurs by loss of the insertion plus one copy of the w^i duplication, restoring gene structure to wild type. This probably occurs by recombination between the flanking copies of the w^i duplication. We have also examined the structure of revertants to w^i by Southern blots of genomic DNA, and have shown, within the experimental limits of ~50 base pairs (bp), that these derivatives arise by excision of the w^c insertion, restoring the w^i duplication (see Fig. 1). However, we could not distinguish between a truly precise excision event and an imprecise event by these experiments. As the revertants do not have a wild-type phenotype, there is no *a priori* reason to assume that restoration of the w^i phenotype requires a precise excision of the insertion.

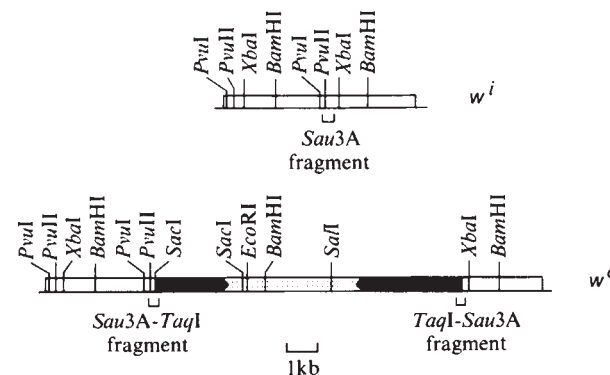


Fig. 1 Sequencing strategy. Restriction enzyme maps of the w^i duplication and the w^c insertion. The sequences duplicated in w^i are indicated by the open bar. Solid bars indicate the terminal inverted repeats of the w^c insertion; the stippled bar defines the portion of the insertion that is not homologous to the inverted repeats. Fragments isolated by molecular cloning for sequence determination are indicated by the brackets below the maps. The left and right termini of the w^c insertion and flanking *white* locus sequences were isolated from plasmids pC10.2 and pC7.6, respectively; each plasmid contains a *Bam*HI fragment corresponding to approximately half of the w^c insertion and some flanking DNA³. As the terminal repeats of the w^c insertion are not cut by *Sau*3A, a large *Sau*3A fragment containing one inverted repeat and its junction with *white* locus DNA was isolated from each plasmid. After digestion, these large *Sau*3A fragments were purified by preparative gel electrophoresis, and digested with *Taq*I, an enzyme which cuts frequently within the inverted repeats of FB elements. The digestion products were cloned into the M13 phage vectors mp8 and mp9, which had been digested with both *Bam*HI and *Acc*I (ref. 19). Hybrid phage DNA molecules were purified and the DNA sequence was determined by the dideoxynucleotide chain termination method²⁰⁻²². The *Sau*3A fragment containing the insertion point in the parental w^i chromosome was isolated from the phage clone λ wⁱB4 (ref. 5). The 2.9-kb *Bam*HI fragment containing the point of insertion was gel-purified from λ wⁱB4, and cloned into the *Bam*HI site of the plasmid vector, pBR322 (ref. 23). The internal *Pvu*I-*Xba*I fragment was then gel-purified from this subclone, and digested with *Sau*3A. The resultant *Sau*3A fragments were cloned into the *Bam*HI site of the phage vector M13mp9 and sequenced as described above. The point of excision was analysed in two independent revertants of w^c to w^i ; these revertants had been analysed previously by Southern blots of genomic DNA³. DNA was isolated from the two revertant fly stocks, digested to completion with *Bam*HI, and ligated into the *Bam*HI arms of the λ vector Charon 28 strain 2221 (ref. 24). The DNA was packaged *in vitro*, and 200,000 phage from each library were screened for homology to the subcloned 2.9-kb *Bam*HI fragment from the parental w^i chromosome described above (see ref. 3 for methods). DNA was prepared from phage containing the homologous 2.9-kb *Bam*HI fragment, and this fragment was subcloned into the *Bam*HI site of the plasmid vector pBR322. The *Sau*3A fragment containing the point of excision was cloned into M13mp9 and sequenced as described above.

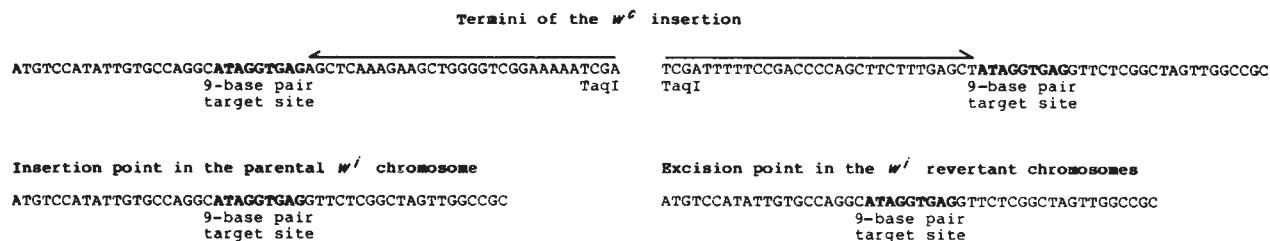


Fig. 2 Sequence of the w^c insertion point in w^i , w^c and two w^i revertants. The 9-bp direct repeat induced by the insertion is indicated by bold type. The first *TaqI* site within the insertion is indicated. All sequences were determined on both strands using the dideoxynucleotide chain termination method²⁰⁻²². The sequence of 100 bp of *white* locus DNA flanking the insertion point was determined in all four chromosomes on both strands; a portion of this is shown here.

Therefore, we examined the nucleotide sequence of the excision point in two independent w^i revertants of w^c to determine whether excision of the insertion is truly precise.

We first determined the nucleotide sequence of the ends of the w^c insertion and of flanking DNA as described in Fig. 1 legend. We next isolated and determined the sequence of a *Sau3A* fragment containing the point of insertion from the parental w^i chromosome (Fig. 1). By comparing these w^i and w^c sequences (Fig. 2), we determined that the w^c insertion occurred 66 bp to the right of the *PvuII* site in the right half of the w^i duplication, and that a 9-bp direct repeat was created as a consequence of the w^c insertion.

Small direct repeats of the target site are caused by the insertion of transposable elements in both prokaryotes and eukaryotes^{1,9}. A 9-bp repeat had previously been detected for another FB element, FB3, by a comparison of sites with and without an element in different genetic backgrounds⁷. However, in this case, one could argue that the duplication reflects a sequence polymorphism between strains, or that the 'empty' site actually resulted from the excision of the element, rather than representing the site before insertion. Our examination of the same locus both before and after a genetically detectable insertion event rules out either possibility. The finding of a 9-bp duplication in two independent cases also suggests that all members of the heterogeneous FB element family make the same size duplication on insertion. A comparison of the two duplicated target sites revealed no apparent sequence homology between them.

A comparison of the right and left ends of the inserted FB element also indicates a high degree of sequence conservation. The 31 base pairs extending to the first *TaqI* sites within the insertion are perfect inverted repeats. Conservation of the ends of FB elements both within and between elements has been detected previously for two other FB elements, FB3 and FB4 (refs 7, 8); the 31 bp at the ends of the w^c insertion are identical to those found at the ends of these elements. Thus, despite the size heterogeneity within the FB family, the ends of FB elements are highly conserved.

We isolated the same *Sau3A* fragment from two independent w^i revertants of w^c to sequence the excision point of the insertion in these revertants (Fig. 1). The sequence of this fragment was identical in both revertants (Fig. 2). A comparison of the sequence spanning the excision point in the two w^i revertants with the same region in the parental w^i chromosome indicates that in both cases of reversion, the w^c insertion has been precisely excised. The entire insertion and one copy of the 9-bp duplication have been removed, restoring the original w^i sequence. This excision is equivalent to a recombination event between the two copies of the direct repeat created by insertion of the element.

Precise excision has been demonstrated for *Tn10* and *Tn5*, two prokaryotic transposable elements which are structurally reminiscent of FB elements^{10,11}. Both *Tn10* and *Tn5* contain long, terminal, inverted repeats, and both create 9-bp duplications on insertion^{12,13}. Precise excision of these elements occurs

independently of transposase functions, and seems to involve the terminal inverted repeats in a mechanism similar to spontaneous deletion formation^{11,14-16}. Precise excisions of the w^c insertion could occur in a similar way.

Alternatively, the high-frequency precise excisions of the w^c insertion could be catalysed by an FB element-encoded 'transposase' function. The dependence of precise excision on element-encoded functions has been observed for another class of *Drosophila* transposable elements, the P elements^{17,18}. Precise excision of these elements occurs only in the genetic condition known as hybrid dysgenesis. In contrast, the instability of the w^c mutation seems to be independent of genetic background. Thus, if a 'transposase' is catalysing this event, it is probably expressed constitutively. Such an enzyme need not even be encoded by the FB elements themselves; the excision of FB elements could be catalysed by other enzymes present in the cell.

The high frequency of precise excisions of both FB and P elements in *Drosophila* distinguishes them from prokaryotic transposable elements for which precise excision is rare, and suggests that there are significant differences between these elements and prokaryotic transposable elements. Transposition mechanisms have not yet been elucidated in *Drosophila* and other eukaryotes, and could involve precise excision and reinsertion of elements at new sites in the genome. Alternatively, if transposition is duplicative, high rates of excision will act to prevent the continuous accumulation of transposable elements within the genome.

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Insulin: passion and the prize

Douglas Black

The Discovery of Insulin. By Michael Bliss.

McClelland & Stewart, Toronto/University of Chicago Press: 1982. Pp.304. \$20.

To be published in Britain in September 1983 by Paul Harris, Edinburgh, £15.

MANY years ago now, I heard Michael Polanyi give his lecture on "Passion and Controversy in Science", later published in the *Lancet* (June 16 1956, pp.921-925). Anyone who doubts his view that scientific debate may be coloured by emotion should read the story of the discovery of insulin, which has now been told by a Canadian professional historian. Polanyi's two elements were fully displayed, together with more than a touch of ineptitude in high places.

However, before looking at the more controversial aspects of the way in which insulin was discovered, and the way in which credit for the discovery was apportioned, it is worth underlining the importance of the discovery itself in the development of medical science. Some 300 years earlier, Harvey had exhorted us to "search and study out the secrets of Nature by way of experiment"; and had himself shown the way in his proof of the circulation of the blood. Between Harvey's day and the present century, much knowledge had accumulated on the natural history of disease, and on the physiology of the body; and of course some empirical remedies such as digitalis had been placed on a sound footing. It was however the discovery of insulin which made it plain to the whole world that physiological knowledge could be applied in such a way as to cure a previously fatal disease. The mould of therapeutic nihilism was broken; the liver treatment of pernicious anaemia followed soon after, and in due course other replacements for vital factors which had been lost.

In the second century AD, Aretaeus of Cappadocia had recognized diabetes as "a melting down of the flesh and limbs into urine". In 1679, Thomas Willis in his account of "the pissing evil" distinguished diabetes mellitus from diabetes insipidus by the "honeyed taste" of the urine. In 1889, Minkowski found that removal of the pancreas in a dog produced diabetes; and in 1900 Opie reported lesions of the pancreatic islets of Langerhans in diabetic patients. In 1905, Starling in his Croonian Lectures had developed the concept of internal chemical messengers, which he named "hormones"; and in 1916 Sharpey-Schafer suggested that diabetes might be caused by a lack of such a hormone. The conceptual stage was thus fully set for the discovery of insulin; and, as described by Bliss, pancreatic extracts of various kinds had been prepared by Paulesco in Romania, Zuelzer in Germany and Scott

and Kleiner in the United States. There was just one problem with all these extracts; they didn't work.

In the autumn of 1920, Frederick Banting in Toronto had the idea that "by the experimental ligation of the duct and the subsequent degeneration of a portion of the pancreas, one might obtain the internal secretion free from the external secretion". He spoke to the Professor of Physiology, J.J.R. Macleod, who rather reluctantly gave him laboratory facilities, and also the help of a student, Charles Best. Work began in the summer of 1921, and is described in detail in Bliss's book, using the original notebooks. There were many difficulties with a high operative mortality, and severe reactions from the crude extracts; but the workers stuck to their task, and eventually it was possible to maintain a pancreatectomized dog for many weeks. It also became apparent that ligation of the pancreatic duct was not necessary, and this of course greatly increased the potential supply of pancreas for extraction. Extracts were first given to diabetic patients in the early months in 1922, but they were very crude — "a thick brown muck" — and they caused fever and other reactions. But they worked. J.B. Collip played an important part in refining the extracts, and thus making the use of insulin in treatment really practical.

Unfortunately, as the importance of the discovery became more apparent, animosi-

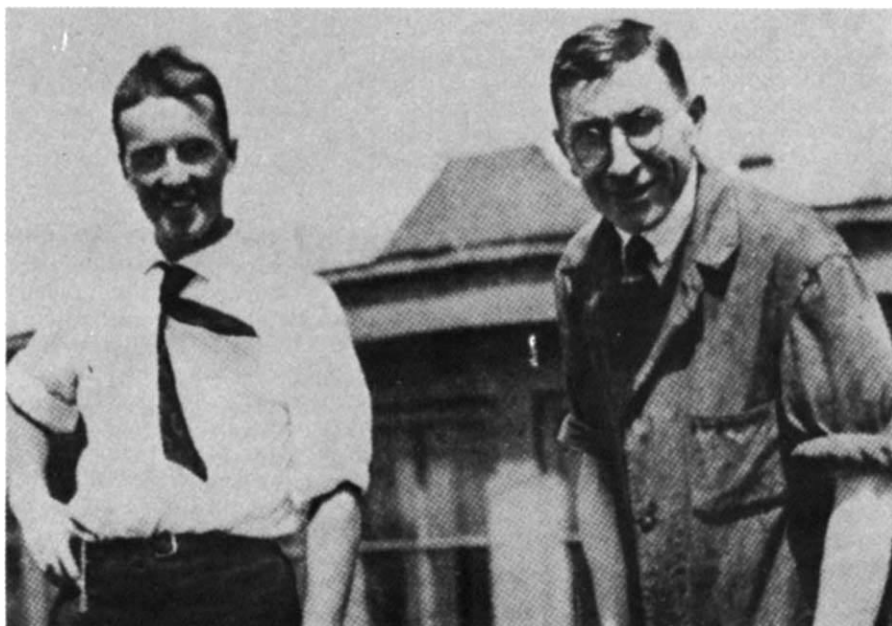
ty among the workers grew. Bliss picks his way carefully through a sequence of acrimonious disputes. Banting suspected Macleod of seeking to obtain the credit, following an occasion at a meeting when a clumsy presentation of the early results by Banting had to be clarified by Macleod from the chair. On another occasion Collip, who had been brought into the team by Macleod, refused to divulge to Banting and Best his methods of purification. There was a considerable quarrel, but in the event Banting, Best, Collip and Macleod signed an agreement. The award of a Nobel Prize in 1923 to Banting and Macleod led to further disagreements, however; Banting at once shared his prize with Best, and Macleod his with Collip. Banting quarrelled at one time or another with all the other three; Macleod seems to have been the most patient. But even he said on one occasion, "If every discovery entails as much squabbling over priority etc as this one has, it will put the job of trying to make them out of fashion".

The final paragraph of Bliss's book puts these matters into perspective, and part of it may be quoted:

Fred Banting, J.J.R. Macleod, Bert Collip and Charley Best knew they were making medical history . . . But perhaps the group at Toronto misjudged both their situation and posterity's viewpoint. They did not realize that those who understand history would eventually come to honour all of them. Above all, we would honour their achievement.

I would strongly recommend this book as a balanced account of a notable medical discovery, which might even be said to mark the dawn of major practical application of physiological knowledge to clinical medicine. □

Sir Douglas Black is Emeritus Professor of Medicine at the University of Manchester.



Making history — Charles Best (left) and Frederick Banting on the roof of the Medical Building at the University of Toronto, in 1921 or 1922.

Up to a frontier of astrophysics

E.N. Parker

Solar Magnetohydrodynamics.

By Eric R. Priest.

Reidel: 1982. Pp.470. Dfl. 235, \$99.

Solar Magnetohydrodynamics was written by Dr Priest over a period of several years as a text for a graduate course in magnetohydrodynamics at the University of St Andrews. In his preface, the author provides an interesting account of the development of the book and puts forward the fundamental point that the Sun, because of its proximity, is the primary astrophysical laboratory for the study of magnetic fields. The fact is, however, that the magnetic fields in the Sun, subject to the convection of the fluid beneath the visible surface, behave in ways that are baffling even to the expert magnetohydrodynamicist. A sunspot is an example of such a mystery, and it is gratifying to see that the chapter on the structure and stability of sunspots includes mention of the current speculation and doubt on the causes of the observed assembly of sunspots from small flux tubes.

The author takes a direct approach to the subject, beginning with the reduction of Maxwell's equations to magnetohydrodynamic form. The remainder of the first five chapters are devoted to the hydrodynamics of electrically conducting fluids carrying magnetic fields, forming the intellectual basis for the applications of that basic physics to the mysteries posed by the Sun and other stars.

The chapter on dynamo theory, attacking the question of the origin of the oscillating magnetic field of the Sun, is lucid and concise, giving a clear overall picture of what is (and is not) currently known about the production of magnetic fields beneath the visible surface. It concludes with a discussion of the outstanding problems with dynamo theory in general, and the solar dynamo in particular, that have yet to be overcome. The account of solar flares is also excellent, as one would expect from an author who has been so directly involved with the theory of magnetic reconnection and the explosive consequences in the solar flare. In the final chapters, Priest goes on to cover the important topics of prominences, and the associated eruptions, coronal transients, coronal heating and the solar wind.

The treatment is both broad and thorough, so that the reader is brought up

Quantum chemistry

Oxford University Press have issued a second edition of *Ab initio Molecular Orbital Calculations for Chemists*. The original book appeared in 1970. Written by W.G. Richards and D.L. Cooper, the new edition costs £8.95, \$16.95 in paperback.

to the working frontier of research. For this reason the monograph belongs on the desk of every astrophysicist interested in active objects, whether stars or galaxies. It should go without saying that the book is also an excellent text for a graduate course on magnetohydrodynamics; it would be relatively easy to base a one-semester course on selected topics, although there is plenty of pertinent material for open-ended study. This subject is, after all, one to which many astrophysicists are, in one guise or another, now devoting their principal research efforts. □

E.N. Parker is a Professor in the Departments of Physics and of Astronomy and Astrophysics at the University of Chicago.

Salt and the scholar

Roger Guillemin

The Hunger for Salt: An Anthropological, Physiological and Medical Analysis.

By Derek Denton.

Springer-Verlag: 1982.

Pp.650. DM360, £85.

The Hunger for Salt is the work of a scientist and scholar. Should one want to read about the endocrine, neuroendocrine and renal mechanisms involved in the homeostasis of sodium, water and related ions, the book contains all that is to be expected from a physiologist as Denton fundamentally is. But there is far more to Denton's treatment of the physiology of sodium and body-fluid regulation to be found in terms of the substantially behavioural stereotype, that of hunger and thirst. Of 10 chapters devoted to such physiological discussions, each is clearly directed at exploring salt appetite as it can be physiologically or experimentally modified.

In each chapter Denton usually ranges far afield in background knowledge, finally to arrive at some basic physiological question. For instance when the question is raised of the neuroanatomical location of the centres that will stimulate or inhibit salt licking or water drinking, Denton's account encompasses the more psychological problem of the animal's conscious awareness of its thirst for water, regardless of the true state of its hydration. Denton does not deal with the neuroanatomy and neurophysiology involved until he has discussed in some depth the general problem of consciousness in animals — citing the studies of Richter in rats, and those of Griffin, Gallup and Thorpe in the great apes — and also man, though somewhat more scantily. Here he quotes Proust (in English unfortunately, but using an excellent and typically Proustian sentence far less well known than that on the taste of madeleines),

Sperry, Hess, Heath and Penfield, without omitting the Popper–Eccles dialogue (with a definite nod to Popper). Broadly, Denton's conclusion is that animals' urgent or chronic appetite for salt must be preceded by true thirst or hunger for salt. A statement such as this, however, is as simplistic an indication of Denton's approach as are complex and extensive the pertinent chapters in the book (which include the presentation of recent studies on the effects of peptide hormones — I prefer the word cybernins — on acute salt appetite in sodium-replete animals).

All of this leads finally to an excellent discussion of the theories regarding the genesis of salt appetite, which culminates in the postulation that activation of salt-appetite drives may involve genetic transcriptions within the relevant neuronal systems, reminiscent of the mechanisms involved in expression of sexual behaviour.

Some of the most unexpected discussions in the book are to be found in the sections dealing with the anthropological background to salt appetite. One chapter, for example, is entirely devoted to cannibalism. Over more than 20 pages Denton gives us a critical survey of reports of cannibalism all over the world, with extracts from Captain Cook's journals, from those kept by Alfred Russel Wallace during his several years in the Amazon basin and also from more recent reports. These writings give eye-witness accounts of endo- or exo-cannibalism, leading Denton to set little store by Ahrens's recent book (*The Man-eating Myth: Anthropology and Anthropophagy*, published by Oxford University Press in 1979). If this extensive treatment of cannibalism is another example of Denton's grasp of anthropology — cannibalism to obtain salt or other minerals, as in the consumption of powdered bones, contributes to the book's thesis only in the context of geographical locations of low-sodium availability — it also shows the importance of hedonism in the hunger for salt.

The final chapter deals with the role of salt intake in the etiology of high blood pressure in modern man. A well-researched 100 pages in which one will find a superb summary of all one could hope to be told on the subject, it critically closes by stating that a causative role of sodium excess in the hypertension of modern Western man has not been unquestionably proven.

The Hunger for Salt is a large book which will not be read from cover to cover, all in one sitting. It is, however, an extraordinary compendium of well-organized knowledge, so rich in its scholarly treatment that it will most certainly constitute a primary source of reference, knowledge and new ideas for anthropologists, physiologists and physicians. □

Roger Guillemin is Chairman of the Laboratories for Neuroendocrinology at the Salk Institute for Biological Studies, La Jolla, California.

The quail's tale

Julian Lewis

The Neural Crest.

By Nicole Le Douarin.

Cambridge University Press: 1983.

Pp.260. £37.50, \$65.

THE neural crest is a most peculiar part of the vertebrate embryo. It originates at an early stage from the region where the neural tube pinches off from the rest of the ectoderm. While the neural tube develops into the central nervous system, the cells of the neural crest break loose from the neuroectoderm and migrate out to form the neurons and glial cells of the peripheral nervous system. This fact, well known since the last century, encouraged the notion that cells with similar origins differentiate in similar ways, and fitted von Baer's theory of the germ layers. But it soon became apparent that the neural crest breaks all such rules: its cells develop not only into neurons and glia, but also, in the region of the head, into almost every sort of connective tissue. Pigment cells also derive from it, as do several types of endocrine cells.

Apart from their initial location, neural crest cells have only one obvious feature in common: they are all migratory. As in the study of any migrant population, the foremost requirement is a method for marking the cells as they set off on their travels, so that they can be identified subsequently. Nicole Le Douarin's involvement with the neural crest began with the discovery of a marking method based on the grafting of cells from quail embryos into chick embryos, where they can be easily distinguished by the appearance of

their nuclei. This simple technical advance opened up for her an Aladdin's cave — or should one say Pandora's box? — of problems for study. What controls the paths of neural crest cell migration? What decides where the cells will halt and settle? When, where and how do the cells become determined for a particular mode of differentiation? Indeed, a whole education in developmental biology could be built around the neural crest, and in exploring the behaviour of its cells one is led into the most diverse fields: from the composition of extracellular matrices to the formation of pigmentation patterns, from the causes of malformations of the face to the biochemistry of neuronal differentiation.

Le Douarin's excellent monograph gives a clear and systematic account of methods for investigating the neural crest, of its migratory properties, and of the development of the various cell types and tissues derived from it. There is a marked emphasis on the findings of Le Douarin's own research group, though the large body of work by other investigators is in general well reviewed. The style inclines more to the careful reporting of facts than to the erection of theories. It is most successful where the facts tell a clear story; less so where there are difficult conceptual problems to be analysed. In particular, I found the discussion of the control of pigmentation patterns somewhat disappointing. But the virtues easily outweigh the defects. The text is a pleasure to read, the illustrations are excellent, and the book has already been practically snatched from my hands for use as a work of reference in the laboratory. □

Julian Lewis is a Lecturer in the Department of Anatomy at King's College, University of London.

Healers of the whole

G. Morris Carstairs

Shamans, Mystics and Doctors:

A Psychological Inquiry into India and its Healing Traditions.

By Sudhir Kakar.

Knopf: 1982. Pp.306. \$15.

SUDHIR Kakar, the author of this set of descriptions of the practices observed in a series of Indian centres for psychic healing, is well qualified for his task. Kakar completed his basic education in India before going on to further training in psychology and psychoanalysis in West Germany, Austria and the United States. While in America he worked for some years in collaboration with Erik Erikson, a psychoanalyst who has paid particular attention to the parts played by contemporary history and culture in personality formation.

In an earlier work (*The Inner World*, published in 1978) Kakar showed great skill

in portraying the essential elements in the major sacred legends of Hinduism, and went on to indicate how such legends continue to influence the behaviour of Hindu men and women to this day. His present book is concerned with healing, and particularly with the healing of mental and psychosomatic disorders, as practised by a variety of traditional healers in India. The setting and mode of practice of each of these healers are described in turn. The first is an elderly, partially-sighted and quite poor man whose consulting room is a small Moslem tomb in Old Delhi. His patients are also drawn from the very poor yet some of them are brought by their relatives from villages many miles away. There is a striking contrast between the squalor of this old man's surroundings and the dignity with he expounds the teachings of Unani, the ancient Arabic school which has descended from that of the Greeks (or Ionians).

The next centre of mental healing considered by Kakar is emphatically Hindu. This is a temple dedicated to

Hanuman in a village a mile or two off the highway which runs from Agra to Jaipur. Here the treatment is conducted by the spirits of Lord Hanuman and those of a number of subordinate deities, contending with the evil spirits which are believed to have been responsible for the patient's malady. This little temple is thronged — especially on Tuesdays and Saturdays when a formal *peshi* or court hearing takes place — by up to 300 patients and their relatives. Many of the patients become possessed by their attendant evil spirits or by Hanuman himself, or one of his assistants. When this happens the relatives engage in conversation with the spirits and are instructed in what they must do.

Similar descriptions follow of the practices of a series of holy men and of one formidable female Guru, Mata Nirmala Devi, who travels the country placing advertisements in the local newspapers announcing her ability to impart instant self-realization to troubled souls.

Each of the descriptive passages is accompanied by a clear outline of the healers' theoretical accounts of the causes of mental disorders, and their cure. Two particularly interesting chapters are devoted respectively to "Tantra and Tantric Healing" and to an account of Ayurvedic theories of mental disease and its remedy. The former discusses an erotically charged philosophy according to which years of self-discipline can be rewarded by a devotee's becoming aware of his true androgynous identity.

As might be expected of a psychoanalyst, Kakar pauses from time to time to take note of his own reactions (whether of attraction or antipathy) to these healers' often forceful personalities. He readily admits that Western science has tended to dismiss Eastern theories about mental illness as unworthy of serious consideration, and does not exempt himself from this criticism. In his chapter on Ayurveda, for example, he writes: "I could not quite summon the required scientific attitude of combined respect and skepticism, an open-eyed wonder and the beady eye of critical doubt which are essential for any serious inquiry".

This book offers a valuable introduction to some of the many forms of traditional healing in India. It is timely because some teachers of Western medicine have made infections and biochemical disorders the paradigms for every form of illness. In many advanced countries there is a renewed interest in holistic medicine as a necessary complement to scientific medicine. Indian traditional medicine has suffered from its lack of scientific inquiry but some of its teachings (including Ayurveda) have long urged the need to consider each patient's whole being and not merely his presenting symptoms. This is surely good practice in any country. □

G. Morris Carstairs was formerly Professor of Psychiatry at the University of Edinburgh.

New Price of medical practice

David Carmichael and Jane Anderson

Oxford Textbook of Medicine.

Edited by D.J. Weatherall,
J.G.G. Ledingham and D.A. Warrell.
Oxford University Press: 1983.
2 volumes, pp.2,700. £45, \$75.

SINCE the publication in 1978 of the twelfth and last edition of Price's *Textbook of Medicine*, which had become rather tired in its old age, there has been considerable interest in what book Oxford University Press would produce to replace it. Despite the criticism that large textbooks rapidly go out of date, there is undoubtedly a place for the successor, the *Oxford Textbook of Medicine* (OTM), both for reference and as a reflection of medical practice in the United Kingdom.

The heart of the book is a systems review of disease, within a framework of anatomy, physiology, biochemistry, epidemiology, clinical presentation, diagnosis (including special investigations) and management. In addition there are separate chapters on basic science covering genetics, immunology and neoplasia. The first chapter, "On Textbooks and Medicine", emphasizes that there is more to being a good doctor than the possession of factual knowledge. The theme is pursued throughout both volumes, being exemplified by the sections on dermatology, psychiatry and terminal care. This last contribution, however, written by Dame Cicely Saunders, would have been better placed in juxtaposition with the first chapter rather than at the end of Vol. 2. The discussions of clinical symptomatology in gastroenterology, respiratory medicine and neurology, with examination laid out on an anatomical basis, should in particular be compulsory reading for both undergraduate and postgraduate students. Even experienced clinicians may find themselves re-evaluating their clinical skills in the light of these chapters.

Both general physicians and practitioners often find it difficult to give clear advice to prospective mothers, either in pregnancy or before. The chapters on reproductive medicine will be helpful and illustrate the value of a comprehensive textbook which is able to include topics which do not fit into one body system. Similarly the relationship of haematology, dermatology and psychiatry to systemic

disease is well presented. An equivalent chapter on the eye would have been useful.

Several topics are covered in two or more sections, and although differences in emphasis are to be expected, some may lead to confusion — prophylaxis of endocarditis, treatment of inappropriate anti-diuretic hormone release, use of acyclovir in chickenpox and diagnosis of *Pneumocystis carinii* are some examples. The formula of arginine vasopressin is shown twice, once incorrectly (the endocrinologist getting it right). Discussion of the management of asthma, in particular the evaluation and treatment of the severe asthmatic, is not clear and may confuse students and some junior doctors. The special investigations for each system may be an area in which the book will date most, with rapid advances in radioisotope, imaging and radiological techniques. In addition, the indications for some investigations are not always clearly set out; for example neither indications nor technique for renal biopsy are discussed satisfactorily. Be this as it may, post-graduates preparing for the examination for membership of the Royal College of Physicians will undoubtedly refer to the OTM and examiners should be prepared to accept information quoted from the book.

The coverage of all aspects of medicine is comprehensive and in this respect the OTM compares favourably with its American competitors such as Harrison's *Principles*

of *Internal Medicine* and Cecil's *Textbook of Medicine*. Inevitably, recent topics such as acquired immune deficiency syndrome have not been included, but there could have been more on Kawasaki syndrome and Staphylococcal toxic shock. The inclusion of a long section on infectious and tropical diseases is wholly justified, both by its high standard and by the worldwide readership at which the books are aimed. It is surprising, therefore, that there is so little on vitamin deficiency diseases in the section on nutrition.

More generally, the lack of an index at the end of the first volume is irritating, even though the way in which the contents are laid out with clear cross-referencing makes the two volumes easy to use. They are, on the whole, well illustrated, although the quality is variable — several radiographs are poorly reproduced and the two pages of colour pictures contribute little.

The requirements of a textbook are that information should be accessible and accurate, and that its extraction should be as stimulating and pleasurable an activity as possible. The editors have certainly met these demands. Any textbook that can make both the serology of syphilis and the classification of porphyria comprehensible must be worth buying. □

David Carmichael is a Senior Registrar in General Medicine and Jane Anderson a student at St Mary's Hospital Medical School, London.

Simple quantum vibrators

R.G. Chambers

The Physics of Vibration, Vol.2, containing Part 2 The Simple Vibrator in Quantum Mechanics.

By A.B. Pippard.
Cambridge University Press: 1983.
Pp.208. £20, \$39.50.

"SIMPLE" is perhaps a relative term, but the first third of this text, at least, should be accessible to the enthusiastic undergraduate. It includes a discussion of the harmonic oscillator (including coherent-state solutions and the driven oscillator), the f-sum rule, anharmonic oscillators and the Bohr-Sommerfeld and WKB approximations (with illuminating examples), and the two-dimensional anharmonic oscillator. It also includes a brief account of the behaviour of electrons in a metal in a magnetic field, including the complications caused by magnetic breakdown: a topic on which the author himself did much of the original work.

The remaining 130 pages are largely devoted to a study, in steadily increasing depth, of a particular model: a two-level system coupled by dipolar interactions to a quantized radiation field and/or to a

classical electromagnetic field. The radiation field is represented by a set of harmonic-oscillator cavity modes, and the two-level system is used to represent, variously, two states of an atom (excited state and ground state), the spin-up and spin-down states of a nucleus in a magnetic field, the even-parity and odd-parity states of a polar group confined to a double potential well in a polymer, and the even-parity and odd-parity states of the NH_3 molecule. The model is applied to a variety of problems — including spin resonance and spin-echo techniques and the stimulated and spontaneous emission of radiation (without recourse to the methods of second quantization) — and the work ends with a study of the ammonia maser.

By this stage, the author can fairly claim to have succeeded in his aim of bridging the gap between undergraduate texts and specialist monographs. But the bridge is a much narrower and more demanding one than it was in Vol. 1, published in 1978, which was devoted to discussion of the simple classical vibrator. In particular the algebraic structure is in places rather dense, and only the most tenacious of undergraduates will fight his way through to the end. Nevertheless, his teacher will find much to ponder on in this original and thought-provoking text. □

R.G. Chambers is a Professor of Physics at the University of Bristol.

Bonner in paperback

Princeton University Press have recently published a paperback edition of John Tyler Bonner's *The Evolution of Culture in Animals*. The book first appeared in 1980 and was reviewed in *Nature* 288, 26 (1980). Price of the paperback is \$9.95, £8.60.

NMR — from chemistry to medicine

Tony Ferrige and John Lindon review the rapid advances that have taken NMR spectrometers from the chemical to the medical laboratory

BEFORE the commercial advent of nuclear magnetic resonance spectroscopy (NMR), about 10-12 years ago, NMR spectroscopy appeared to have found its niche. Spectrometers were being used in both academic and industrial chemical laboratories to study relatively simple molecules whilst the large biological molecules were considered too complex for the technique. Since then, however, the widespread introduction of NMR systems using Fourier transform techniques has led to an explosion in the applications of NMR. Due largely to simultaneous improvements in the two main elements of the NMR system — the data acquisition technology and the data processing techniques — NMR can now be used to study biochemistry, physiology and medicine.

NMR relies on the interaction between magnetically sensitive nuclei which are exposed to both a strong magnetic field and a pulsed radio frequency. Almost all elements have a magnetically active isotope which can be observed by NMR. The most readily detected, and hence the most frequently studied, is the proton, ^1H , but other less sensitive, although commonly used, nuclei include ^{31}P , ^{13}C , ^{15}N and ^{17}O . It is the development of more sensitive techniques coupled to advances in data analysis to reduce the background noise associated with biological molecules which has made the analysis of whole organs or even live animals now possible.

Technical advances

On the technological front, helium-cooled superconducting magnets are now commonplace. These boost the magnetic field obtained from the equivalent of 100 MHz for ^1H which is produced by a conventional iron magnet to a commercial limit of 500 MHz achievable with a superconducting magnet.

On the data analysis front, computing hardware has advanced dramatically.

Multi-tasking virtual memory computers coupled to array processors now perform Fourier transforms in a few milliseconds, and whereas before data acquisition and analysis had to be carried out separately, they can now be run concurrently. Previously, analysis required a mini-computer, but there is a growing trend for these to be replaced by microcomputers with an array processor option. Programs are increasingly written in high-level languages so that they can be modified by the user, and automatic processing routines, such as spectrum phasing, are being introduced.

Specific advances

In conjunction with these non-specific technical advances which have had an indirect impact on NMR, the technique has benefitted enormously from improvements in specific areas. The development of multiple pulse sequences has allowed even the most complex spectra to be simplified so that large biological molecules can be studied. Similarly, the advent of fast 15 and 16 bit analog to digital converters, as compared to the conventional 12 bit models, has increased the amount of data which can be processed and, coupled to the use of good non-spinning resolution solenoid magnets, has opened the door for on-line HPLC-NMR using flow cells. This was previously impossible because of the strong masking signal derived from the chromatography solvent. The development of high-resolution non-spinning techniques, facilitated by the high power superconducting magnets, has eliminated the spinning side-bands which formally confounded the interpretation of quantitative ^1H measurements.

Perhaps the most important development however, has been the extension of NMR techniques to the solid state through high-resolution ^{13}C solid state NMR. This

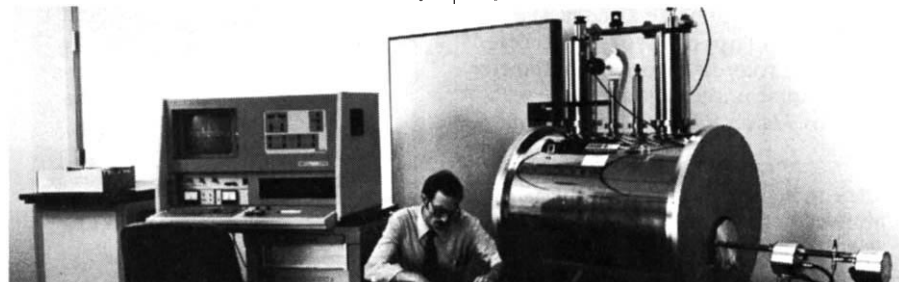
technique, the result of extensive collaboration between industry and university, is now available commercially. It uses a special pulse sequence which transfers the high ^1H sensitivity to the ^{13}C nuclei whilst removing ^1H - ^{13}C interactions. This high sensitivity coupled to a high sample spinning rate (about 250,000 r.p.m.) at a very precise angle ($54^\circ 44'$) relative to the magnetic field yields narrow signals which gives the technique further gains in sensitivity. The resulting sharp spectra compare with those obtained from solutions and allow the extraction of information on molecular conformation and interactions.

Biological applications

Using these new techniques and the vastly improved multi-dimensional data display options provided by commercial colour raster visual display units, NMR is now used to extract information on molecular conformation and interactions including transport through cell walls and tissues.

NMR has now entered the biological and medical laboratories, where it is used to study both isolated tissues and whole limbs or organs. It is used to predict the viability of human kidneys prior to transplantation and to trace the metabolism of labelled substrates or the effect of drugs on metabolism. For the study of organs and limbs within whole animals a new technique, topical magnetic resonance, has been developed which uses field gradients and complex pulse sequences to obtain spectra, usually ^{31}P , by focusing in on any organ in the body. For humans, special magnets with bores of 20-60 cm (compared to a conventional 49-110 mm) have been designed to accommodate whole limbs. Other related systems which measure proton density and relaxation times are now commercially available for use in tumour and cancer detection.

NMR is cheaper now in real terms than at any other time, both in cost per Hertz and in the number of experiments that can be done on even standard instruments. As the past decade has seen the demise of non-Fourier systems so the next decade should see the further expansion of the applications as field strengths, and hence frequencies, increase allowing alternative detection methods. □



The Nicolet S-100 NMR spectrometer for ^{13}C solid state analysis

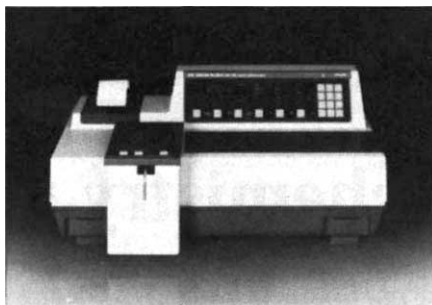
Tony Ferrige and John Lindon are from the Wellcome Laboratory, Beckenham, UK.

UV/visible spectro-photometers

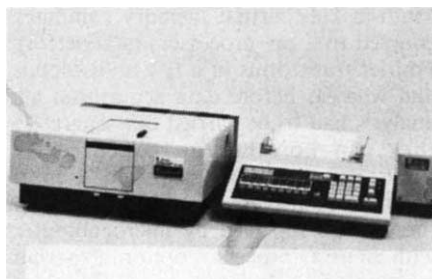
● Du Pont has introduced a microprocessor-driven UV spectrophotometer with digital electronic design for use in HPLC. The detector is the newest addition to Du Pont's 8800 series liquid chromatography system. The instrument has two new features: a new optical section, which incorporates a deuterium light source and an optimized flow cell, and the treatment of digital signals by the microprocessor. In addition four options made possible by the microprocessor and specialized software increase the number of functions that the detector can perform to include automatic wavelength scanning, wavelength/time programming, the ability to obtain absorbance ratios at two wavelengths, and a general-purpose IEEE interface bus. The new spectrophotometer also has a self-diagnostic capability which will monitor, for example, the critical operating characteristics of the deuterium light source, and check for proper voltage and current. The instrument has a photometric drift of 0.002 AU per hour at constant temperature. The temperature sensitivity coefficient is 0.00005 AU per °C and the detector's wavelength range is 195–600nm; its wavelength accuracy is ± 2 nm. *Circle No. 110 on Reader Service Card*

● The DW-2C double-beam, dual-wavelength UV/visible spectrophotometer from SLM Instruments is based on the Aminco DW-2 and DW-2a spectrophotometers and contains built-in data processing capabilities. These provide digital readout, automatic baseline correction, derivatives of spectra and kinetic reaction data, 14 spectral storage locations, memory display on any oscilloscope, and allow the plotting of acquired data on a built-in recorder. Also provided are capabilities to do digital filtering, smoothing, averaging and logarithms, as well as the ability to store kinetic reactions of milliseconds to 25 minutes full scale. The instrument can be operated in several different modes, including double-beam, dual-wavelength, dual-wavelength scanning, rapid kinetics, and user-definable x-axis modes. *Circle No. 111 on Reader Service Card.*

● Perkin-Elmer has introduced an external integrating sphere as an accessory for their UV/visible spectrophotometers, the models 550SE, 551S, Lambda 3 and Lambda 5. With this new accessory, it is possible to collect and measure the light from samples that are remote from the UV/visible spectrophotometer, without any destruction of the sample, using fibre optics and an integral end-on photomultiplier. *Circle No. 112 on Reader Service Card.*



Pye Unicam's PU 8600



The UV 240 from Shimadzu

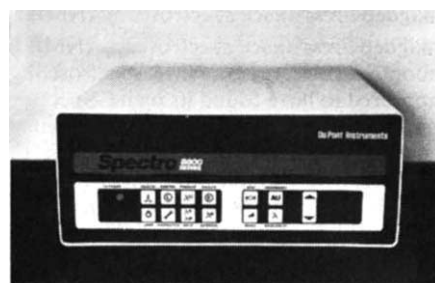
● Pye Unicam has introduced the PU 8600 series of microprocessor-controlled UV/visible spectrophotometers. This is headed by two main versions — the PU 8600 model for industrial and teaching applications, and the PU 8610 model which is designed for the life scientist. The PU 8600 series complements the SP 6 series which is already on the market. Features common to the two ranges include an optical system based on a master holographic grating and a large cell compartment. The PU 8600 series can store up to 10 programs and is supplied with a printer/plotter. *Circle No. 113 on Reader Service Card.*

● A range of spectrophotometric instrumentation is now being manufactured by the Japanese company Shimadzu, and is being distributed in the UK by V.A. Howe. The range includes UV/visible models, IR spectrophotometers, such as the IR 408, and a microprocessor-controlled atomic absorption spectrophotometer. The largest number of instruments cover the UV/visible spectrum. The most simple of these are the UV 120-02 and the UV 120-01 which have single silicon photocell detectors to reduce levels of noise and drift. A four-position cell changer is mounted in a cartridge-style compartment to allow easy removal for cleaning. The UV 150 is the next instrument in the range and is a double beam, digital UV/visible or visible-only spectrophotometer. At the top of the range is the UV 240, a microprocessor-controlled UV/visible recording spectrophotometer with a graphic printer/plotter. *Circle No. 114 on Reader Service Card.*

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



Hewlett Packard's HP 8451A



Du Pont's UV spectrophotometer

● Hewlett-Packard's new HP 8451A diode-array spectrophotometer is a computer-controlled system capable of generating spectra over a wavelength range of 190 to 820 nm in 0.1 seconds. The HP 8451A has an interactive keyboard control and basic programming. A sipper system and automatic sampler can be added. In addition, results can be displayed on the instrument's built-in CRT and printer/plotter. The HP 8451A is for use in time-based analyses such as kinetics experiments and liquid-chromatography detection and can measure repetitively 25 single wavelengths every 0.1 second. Other features of the system include five concentration routines (four single and one multicomponent) and matrix, multi-component analysis for quantitation of mixtures with up to 12 components. The HP 8451A has two levels of data-handling capability. The first is accessed from the standard functional keyboard with key-stroke commands and the second is achieved by adding an optional alphanumeric keyboard. With this second keyboard, basic programs can be developed which function interactively with instrument measurements and which tailor the analysis to specific applications. The keyboard also allows access to the HP-85 personal computer. *Circle No. 115 on Reader Service Card.*

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Lesion location by temperature difference is now possible. Model TH-6D has 1/100° resolution and spots minute differences not previously measurable.

Range: -10 to +60°C $\pm 1/10^\circ$. Measures differences to 1/100°C.

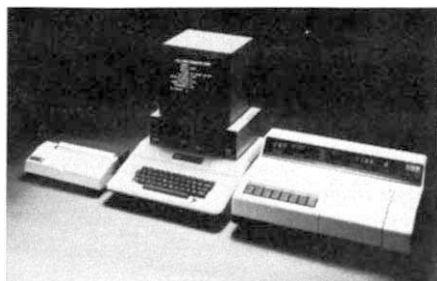
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Cecil Instruments' single instrument



The LKB Ultrospec and Apple II system

● Cecil Instruments have just launched a new series of six single beam spectrophotometers which includes both UV/visible and visible instruments. The equipment has a choice of analog or digital read-out and the digital read-out versions allow for electronic auto-zero of absorbance and computer coupling. A range of accessories is also available which includes thermoelectric, electric and water temperature control, a sipper or cell pump system for rapid sampling, four cell programming and a variety of cell holder options. Printers, plotters and several interface configurations are also available.

Circle No. 116 on Reader Service Card.

● LKB have introduced a line of Apple-based software packages. These are designed to extend the use of their Ultrospec series of single-beam spectrophotometers to include analyses generally carried out by double-beam instruments. This is made possible by using the Ultrospec equipment in conjunction with an Apple II Plus micro-computer and the associated software and interface packages. Software packages are currently available for performing wavelength scans and reaction rates. The wavelength scan package contains programs for wavelength analysis and multi-point calibration as well as the basic wavelength scans program. The wavelength scan program offers sample scan with baseline and reference correction, baseline store facility for successive sample scans, first and second derivative spectra, the option to select and display an area of the spectrum with choice of scale expansions, and plot offset. The reaction rate package is for determination involving reaction kinetics and offers timed monitoring of the rate of change in absorbance. It will plot the results and also calculate the activity or concentration by an entered factor.

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Spectrometers

● Three new accessories which extend the capabilities of the Baird plasma/AFS atomic fluorescence spectrometer have been introduced by Baird-Atomic. A continuous-flow hydride generator, which permits the simultaneous determination of hydride and non hydride forming elements, is now available, along with argon-purged detection modules for phosphorus and sulphur. These modules extend the number of elements which can be routinely analysed to 67. Another development is a hydrofluoric acid resistant torch with a central carbon tube. This feature extends the life of quartz torches while allowing the analysis of samples which require dissolution in concentrated hydrofluoric acid.

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● EDT Research have extended their range of analytical instrumentation by introducing two new spectrofluorimeters, which are manufactured by Jobin-Yvon, to complement their existing JY3 models. In addition to the features of the current JY3 series, the new JY3C model has a computer-compatible scanning unit programmer. This has a digital readout with manual and automatic zero adjustment, a stepping motor for each monochromator, and a control console and power supply offering scanning in 2 directions, 10 scanning speeds and up to 99 scanning cycles. The model also includes a four-cell sample compartment that can be thermostatically controlled, with a magnetic stirrer for the measurement cell. The second new model from EDT Research, the JY3CS, incorporates a microcomputer in addition to the optical unit. The 8-bit Silex microcomputer with 48 kbytes of memory is supplied with spectrofluorimetry software. This provides plots of excitation, emission and synchronous spectra, 3-dimensional spectra and quantum yield measurements. It performs mathematical operations and stores and saves spectra. The JY3CS can also be connected to a Bryans Computagraph plotter for A3 format and to an HP 7225 plotter for A4 format.

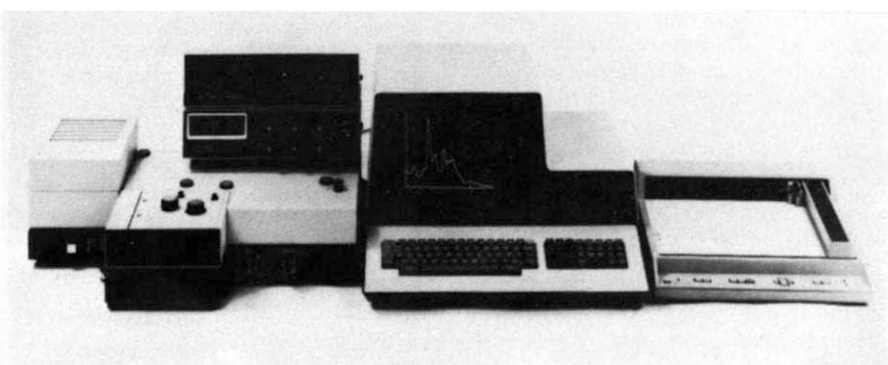
Circle No. 119 on Reader Service Card.

● The analytical capability of Philips' PV 8350 modular vacuum emission spectrometer system has been extended by the introduction of a number of new features and accessories. For example, it is now possible to measure the lines Na λ 488.9 nm and Li λ 610.3 nm in the first order. Exit slits for these lines are mounted on the Rowland circle on the opposite side of the entrance slit to the normal exit slit block. This avoids compromises associated with expanding the wavelength range by measuring lines in higher orders. For metals analysis, a new 50 Hz source unit — the PV 8560 — is also available for installations where the very high speed of the Philips PV 8530 500 Hz source is not required. In addition, the spectrometer can be delivered with the PV 8470 glow discharge lamp, which is suitable for the analysis of thin sheet metals and for the study of elemental concentrations as a function of sample depth. Several new accessories are announced for inductively coupled plasma versions of the PV 8350. These include automatic background correction and (according to the computer configuration) a choice of automatic systems for handling batches of solutions, for the dilution of samples or for measurement by multi-element standard additions.

Circle No. 120 on Reader Service Card.

● Finnigan MAT has introduced the 8500 series gas chromatograph/mass spectrometer (GC/MS). This new GC/MS will perform both high- and low-resolution analyses and its analytical capabilities support analysis of complex high molecular weight samples of various compound classes. Optional equipment, such as a jet separator for packed columns, collision activation chamber in the second field-free region, liquid chromatography/mass spectrometry and an automated direct evaporation probe extend the analytical capabilities of the 8500 series. The analyser is of reverse Nier-Johnson design, which allows metastable ion analysis in both the first and second field-free regions. The magnetic sector of the 8500 is a fully laminated H-shaped magnet for high scan speeds and dynamic focusing. Scan speed is 0.1 seconds per decade up to 1,000 seconds per decade in 24 steps.

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The JY3CS from spectrofluorimeter available from EDT Research

Spectrometer systems and accessories

● Perkin-Elmer's new inductively coupled plasma (ICP) emission spectrometer, the ICP/6000, is designed for multi-element trace metal analysis. Controlled by Perkin-Elmer's new laboratory computer and dedicated ICP software, the system is capable of analysing samples sequentially at 20 elements per minute. The ICP/6000 has a dual-grating monochromator to increase resolution over the wavelength range 175 to 900 nm. Each grating is used only in the first order. The optical system can be purged with an inert gas for analyses in the far UV region. The complete sample introduction assembly of the ICP/6000 is resistant to corrosion so that the system can be used to aspirate any laboratory acid, as well as high levels of dissolved solids. The plasma torch is demountable with a choice of quartz or alumina sample aerosol tubes. *Circle No. 122 on Reader Service Card.*

● Tracor Analytic's GammaTrac 2250 spectrometer is designed for use in physiological research involving radioactive microspheres in circulatory investigations. The system provides the final perfusion rate results in milliliters per minute per unit weight. GammaTrac 2250 has a microprocessor-based multi-channel analyser which accumulates the spectrum of each sample and stores the data in the memory for processing. Accumulated data can be read out on a channel-by-channel basis within selected regions of interest. Sample changing is carried out by means of an automatic, reversible, serpentine conveyor. An optically-controlled elevator positions each sample in the true-well sodium/iodine crystal detector. Massive lead shielding is present around the detector. Standard readout is through a built-in CRT display and optional readout devices include an external printer and x-y recorder. Among other options are two floppy disk packages for permanent data storage, Flextran data processing software, and an on-line Mettler balance. *Circle No. 123 on Reader Service Card.*

● Baird Corporation has introduced AIDS, an automatic injection and dilution system for the inductively-coupled plasma analysis of viscous fluids. This automatically dilutes samples immediately before nebulization so that the Baird Plasma Spectrometer can analyse 60 elements in 80 samples (4,800 elemental determinations) per hour. *Circle No. 124 on Reader Service Card.*

● The LR-36 from Applied Photophysics is a computer-controlled Raman spectrometer which uses a multichannel detector for high speed analysis. The spectral plane can be zoomed to provide variable resolution and frequency range at high sensitivity.

Circle No. 125 on Reader Service Card.

● LKB has introduced the 4070 Autofill "sipper" accessory, designed specifically for the UltroSpec 4050 and 4051 to provide a complete system for the measurement of a large number of samples. The UltroSpec six-sample carousel can be replaced by the Autofill's built-in peristaltic pump and flow-through cell, thereby automating the filling and emptying of the cell. Measurement of absorbance, percent transmittance or concentration can be made at a preselected time interval or at repeated intervals until completion of the reaction. The Autofill can return the sample to the original tube or deliver it to a waste container. A wash cycle simplifies flushing of the flow-cell. The LKB Autofill is available with or without a Peltier-effect thermostatted cell holder and the thermoelectrically heated cell may be set to any temperature between 20 and 60°C or to one of five preset temperatures: 20, 25, 30, 37 and 45°C. *Circle No. 126 on Reader Service Card.*

● A book which will provide an introduction to inductively coupled plasma (ICP) atomic emission spectrometry is to be published in July. "A Handbook of ICP Spectrometry" by Michael Thompson and Nicholas Walsh will combine a review of the theory of ICP with a discussion of some of its principal applications, concentrating on how the technique can be applied to the geological and environmental sciences. *Circle No. 127 on Reader Service Card.*

● A new 400 MHz superconducting Fourier transform nuclear magnetic resonance spectrometer, the XL-400, has been launched by Varian Associates. This new research instrument, known as the XL-400 superconducting FT NMR spectrometer, has a ^{13}C sensitivity per unit volume which is independent of magnetic field strength. The XL-series includes software, particularly the pulse sequence generation (PSG) capabilities, which allows 1 and 3 dimensional experiments to be performed. It is written in Pascal and provides message displays, parameter calculations and branch points.

Circle No. 128 on Reader Service Card.

● Oxford Instruments has produced two new magnet systems — a high-field superconducting magnet with two separable sections, and a 9 Tesla split-pair magnet with a dilution refrigerator. The superconducting magnet will give fields of 13 Tesla and can be used for solid state NMR experiments. It has an outer niobium titanium magnet which provides the centre field contribution of up to 8.5 Tesla, while an inner coil section wound from higher performance niobium tin superconductor provides the additional field strength. The fact that these two sections can be separated using soft soldering techniques means that the magnet has three options — an 8.5 fully persistent magnet with a 75 mm cold bore, a 12 Tesla magnet with a 35 mm bore which is held at 4.2 K, or a 13 Tesla magnet which is at 2.2 K.

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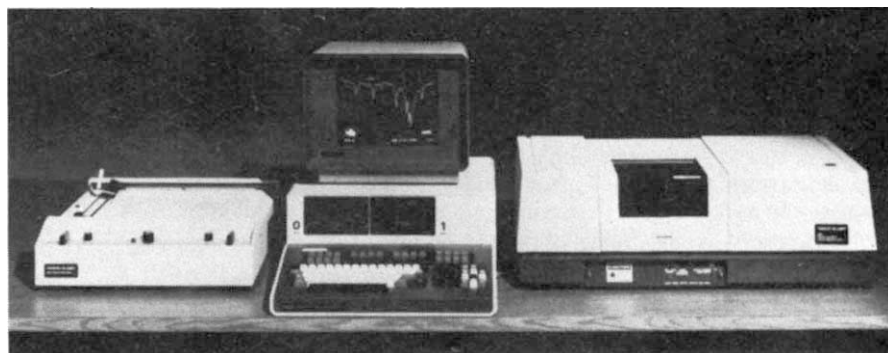
Fourier transform IR spectrophotometers

● The model 1500 Fourier transform infrared (FTIR) spectrophotometer system from Perkin-Elmer has a dedicated Fourier transform processor which calculates an IR spectrum in 0.2 seconds transform time, and presents a video display of the complete IR spectrum in 12 seconds with a resolution of 2 or 4 cm^{-1} . The 1500 FTIR system uses Perkin-Elmer's 3600 IR data station to control the interferometer and to implement the processing of IR spectral data. Applications software for qualitative and quantitative analysis is also available.

Circle No. 130 on Reader Service Card.

● Accuspec have introduced a new gas chromatography/infrared package for Fourier transform IR spectrophotometers. The package consists of the model 66 heated cell, the Accuspec Compact gas chromatograph, IR with micro-thermal conductivity and dual flame ionization detectors, auto-cooling linear temperature programming, and capillary systems, and the model 66 light pipe and heated transfer system. The light pipe is a one-piece construction in stainless steel, rated to 270°C, and is fitted with potassium/bromide windows.

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Perkin-Elmer's model 1500 FTIR spectrophotometer